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The Influence of Vehicles on the Action of Drugs*

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The influence of such vehicle ingredients as sucrose, dextrose, glycerin, propylene glycol, and alcohol on the gastrointestinal absorption of sodium pentobarbital and metrazol has been studied in mice. Sucrose, dextrose, and glycerin in concentrations above 10 per cent inhibit the absorption by a purely local mechanism. Alcohol in concentrations of 10 per cent or more enhances the action of sodium pentobarbital and decreases the metrazol action, both actions being systemic. Propylene glycol exhibits the qualitative effects of alcohol, but is quantitatively less active. When glycerin, alcohol, and propylene glycol are administered intravenously, systemic forces interfere with the absorption of sodium pentobarbital and metrazol although following oral administration the actions are not apparent. When a drug is dissolved in a vehicle composed of two or more ingredients, mutually antagonistic, the degree of antagonism is dependent upon relative concentrations.

PHARMACY has long been interested in the development of appropriate vehicles for the oral administration of drugs, and that a great deal of product improvement has been made may be seen by reference to any of the standard formularies. Most of the improvements have been in the stability, compatibility, palatability, and pharmaceutical elegance of the finished product. In comparison with these factors relatively little attention has been devoted to the effect that the vehicle may exert on the action of the drug. A survey of the literature does not reveal a study undertaken from the point of view to be presented below although there are studies on the absorption from the gastrointestinal tract which have a bearing on the problem.

Seeberg and Dille (1) and Seeberg (2) in studies on the gastrointestinal absorption of barbiturates were not primarily interested in the vehicle, although Seeberg did improve the absorption of barbital by eliminating glycerin from the elixir and using sodium barbital instead of barbital. These authors used three criteria in evaluating absorption: the degree of depression produced by the barbiturate being studied; a chemical analysis of barbiturate blood levels; and a chemical analysis of gastrointestinal residue, all determined at a fixed half-hour period

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after administration. Dille and Whatmore (3) made use of two other methods in studying the absorption of Lanantoside C from various portions of the intestine, namely, essential absorption and biological assay of gastrointestinal residue after a fixed period of time. Other representative studies bearing on the subject have described the effect of the concentration of solute on absorption from the same vehicle (4), the action of vehicle ingredients on isolated intestinal loops (5), the effect of drugs on the emptying time of the stomach (6, 7), and the effect of tonicity (8, 9) and fasting (10) on absorption. Most, if not all, of these methods have in common conditions which make it difficult to correlate the results with action in the normal intact animal. Perhaps most important of these conditions is that the studies were not undertaken for the express purpose of evaluating differences in the vehicle. Also that they involve anesthesia, surgery, and, frequently, sacrifice of the animal at a definite time interval. These conditions are conducive to the use of a relatively small number of animals and do not afford opportunity to study the pharmacological interaction of the drug and vehicle, particularly the duration of action.

From a practical standpoint there are several obvious factors which may influence the action exhibited by a given drug in a specific vehicle. Some of these are as follows: pharmaceutical incompatibility, chemical interaction of the drug and vehicle, pharmacological antagonism or synergism of drug and vehicle, a change of absorption rate from the gastrointestinal tract and toxicity of the vehicle itself. The following experiments were designed to study some of these influencing factors.

EXPERIMENTAL DESIGN

Adult mice weighing between 20 and 30 Gm. were used for the major portion of these studies because they permit multiple observations of relatively large numbers of intact animals, and either oral or intravenous administration is easy and convenient. The mice were maintained on an adequate diet and were not fasted before use. A period of at least one week was allowed to elapse between experiments on any group of mice. There was no significant sex difference following oral administration of the drugs and the oral results were therefore combined and only females were used in subsequent experiments.

Since analysis, either chemical or biological, does not necessarily reflect the action level of the drug, it is obvious that the use of an easily observed pharmacological end point would have definite advantages in this study. The two drugs used were selected for their ability to exhibit such an end point following either oral or intravenous administration, for their known capacity to interact with other drugs, and for their solubility in the various vehicles to be studied. The first of these drugs was sodium pentobarbital with loss of righting reflex for an end point, and the second was metrazol with the incidence of convulsions, sufficiently violent to cause loss of balance, as the end point.

Aqueous solutions of the drugs administered orally and intravenously were used as the standard for comparison of activity. Percentage dilutions of the vehicles, and the administration of the drugs unless otherwise qualified, implies "in aqueous solution," although this term is omitted to avoid repetition. The following vehicles were selected as representative of the class and as those which are most commonly used: 10, 25, and 50% aqueous solutions (*v/v* or *w/v*) of sucrose, dextrose, propylene glycol, and glycerin, and 10% (*v/v*) alcohol, for oral administration. In order to approximate empirically the vehicle ingredient level in the blood following oral administration, a 10% concentration was selected for intravenous administration. In a few cases this was increased to 25 and 50%. While in practice the percentage concentration of ingredients of vehicles varies considerably, and in many instances two or more of the ingredients are combined in a single vehicle, it would be impracticable to study all of the possible concentrations and combinations experimentally. The dosage of the two drugs employed in these studies is far in excess of those employed therapeutically; therefore it was necessary to employ a high concentration of each vehicle ingredient in order that it, too, might exert a maximum effect. The net result from such a combination of high concentrations should approximate as nearly as experimentally possible the influence exerted by vehicle ingredients in lower concentrations on the action of drugs in therapeutic doses. The concentration of the drugs in the vehicle was adjusted in such a manner that the dose to be administered, while on a mg./Kg. basis, was limited in volume to from 0.40 to 0.60 cc. and within this limit varied according to the weight of the animal. The total volume of the intravenous dose was limited to between 0.20 cc. and 0.36 cc.

In control experiments using the vehicle with no added drug the oral administration of the standardized dose caused some symptoms of discomfort following the more concentrated solutions, but none that would be confused with the drug end point except as follows. High concentrations of glycerin and propylene glycol caused diuresis and a peculiar type of convulsion. Concentrations of alcohol

greater than 10% were not used because the standardized volumes employed produced observable pharmacological effects without the addition of other drugs. Intravenously 10% alcohol caused loss of righting reflex in 50% of the animals, while 25% glycerin or propylene glycol caused diuresis and the convulsive movements mentioned above.

The oral ED_{50} (effective dose for approximately 50% of the animals) in aqueous solution was established for each of the drugs. This dose was then administered orally in the several concentrations of each of the vehicles. If the results following administration of these doses were significantly different from those following the control, the dose of the drug was varied in order to establish an ED_{50} for the particular vehicle involved. It was thus possible to estimate the influence exerted by the vehicle in terms of percentage of the aqueous dose. This same procedure was repeated for the intravenous route of administration. Since the intravenous administration circumvents the chemical action of the gastrointestinal tract and the factor of absorption, it was possible to determine pharmacological interaction as distinct from either of the above two factors. In another series the animals were given the vehicle orally and after a lapse of time comparable to the time of onset following the administration of the drug orally, the drug was given intravenously. This procedure was reversed in a group of mice which were given the vehicle intravenously followed immediately by the oral drug in aqueous solution. The mortality in these experiments was not significant except as noted in the tables.

While such an experimental design has certain obvious shortcomings, and the present studies are limited to two drugs, it is believed that the results obtained serve to establish a general pattern and a basis for further investigation. The principal concepts involved are a pharmacological end point, an adequate number of tests, a method for studying the type of influence exerted by the vehicle, the use of intact animals without fasting, and the observation not only of incidence of action but also onset time, duration of action, and mortality.

Other experiments designed to elucidate the various problems arising during the course of the procedures described above are discussed in the text of this paper.

RESULTS

The results from the principal experiments outlined above are summarized in Tables I and II. For convenience of interpretation the results obtained with each vehicle are discussed below:

Controls.—The evaluation of the influence exerted by the vehicle is based on the following established ED_{50} for each drug in aqueous solution: Orally—sodium pentobarbital 50 mg./Kg., metrazol 100 mg./Kg. Intravenously—sodium pentobarbital 30 mg./Kg. and metrazol 20 mg./Kg. Other doses summarized in the tables are for the sake of comparison with results from different routes of administration and vehicles. All exploratory doses have been omitted in the interest of brevity.

Sucrose.—There is no significant difference between the effect of either drug in aqueous solution and its effect in a 10% sucrose solution. Twenty-five per cent sucrose solution interferes with the absorption of sodium pentobarbital but not of metrazol. The ED_{50} increases 100% for sodium pentobarbital and 20% for metrazol when these drugs are administered in 50% sucrose (Table I). That interference with absorption is the mechanism involved in this decreased potency is established (1) by the fact that intravenous administration of either drug in 10% sucrose solution does not materially affect its action (Table I), nor does any combination of oral and intravenous administration markedly influence the results (Table II). Since there seems to be no synergism or antagonism, and the systemic action of the intravenously injected sucrose does not interfere with absorption of the drugs, the interference with absorption following oral administration appears to be a local phenomenon due perhaps to hypertonicity.

Dextrose.—The administration, orally or intravenously, of either drug in a 10% dextrose solution does not materially alter its action. The ED_{50} increases 100% for sodium pentobarbital and 25% for metrazol when these drugs are administered in 50% dextrose (Table I). The data indicate that this decreased action is due to interference with absorption in the manner described for sucrose.

Propylene Glycol.—Concentrations of propylene glycol greater than 10% synergize with sodium pentobarbital and antagonize metrazol following oral administration. Data in Table I indicate that this interaction amounts to approximately 40% for either drug. Intravenously the synergism between propylene glycol and sodium pentobarbital is readily apparent, but since the propylene glycol alone administered intravenously tends to produce a convulsive state, the antagonism with metrazol is difficult to evaluate. This interaction between propylene glycol and the two drugs continues to be apparent when propylene glycol is given orally and the drugs are given intravenously (Table II). When the propylene glycol is given intravenously and the drugs orally there is a decrease in the potency of both. It appears that this is due to a systemic action of the propylene glycol which is not sufficiently strong to be observed when the vehicle and drug are administered together orally.

Glycerin.—Concentrations of glycerin in excess of 10% administered orally interfere with the absorption of both sodium pentobarbital and metrazol. The ED_{50} increases 100% for sodium pentobarbital and 25% for metrazol when these drugs are administered in 50% glycerin. Intravenously 10% glycerin exerts no appreciable effect on either drug, but 25% glycerin increases the incidence of end point and produces a high degree of mortality with both drugs. The separate administration of glycerin and

TABLE I—INFLUENCE OF VEHICLES ON THE ACTION OF SODIUM PENTOBARBITAL AND OF METRAZOL ADMINISTERED ORALLY AND INTRAVENOUSLY

			Sodium Pentobarbital					Metrazol			
Vehicle	%	Route	Dose, Mg./Kg.	No. of Mice	Righting Reflex Loss, %	Onset Time, Min.	Duration, Min.	Dose, Mg./Kg.	No. of Mice	Convulsions, %	Onset Time, Min.
Water	..	Oral	30	10	0	..	..	100[a]	100	47	5.7
	..	Oral	50[a]	60	52	6.0	38.6	125	10	80[b]	2.7
	..	Oral	100	10	100	3.0	130.0	150	10	90[b]	1.6
	..	Vein	27.5	10	0	..	..	19	10	20	0.0
	..	Vein	30[a]	24	58	1.1	10.5	20[a]	..	55	0.0
	..	Vein	32.5	10	60	2.2	3.4	22.5	10	70	0.0
	..	Vein	35	5	100	0.9	14.1	..	..	..	..
Sucrose	10	Oral	50	60	35	7.4	17.9	100	60	55	5.2
	25	Oral	50	60	5	6.5	20.0	100	60	65[b]	6.4
	50	Oral	50	60	10	7.9	22.0	100	60	20	9.0
	50	Oral	100[a]	10	50	5.0	65.0	120[a]	10	60	6.1
	10	Vein	30	20	25	2.3	3.4	20	20	15	0.0
	10	Vein	32.5[a]	15	53	2.4	4.3	22.5[a]	15	53	0.0
	25	Vein	30	10	60	1.2	5.6	..	..	..	..
	50	Vein	30	10	60	0.0	8.9	..	..	..	..
Dextrose	10	Oral	50	30	53	7.1	16.9	100	30	47	2.7
	25	Oral	50	30	10	7.3	24.6	100	30	23	6.6
	50	Oral	50	30	0	..	..	100	20	15	9.1
	50	Oral	100[a]	10	60	5.5	38.0	125[a]	10	60	8.3
	10	Vein	30	20	35	2.3	14.0	20	10	70	0.0
	10	Vein	32.5[a]	10	50	1.8	9.7	19[a]	10	40	0.0
Propylene Glycol	10	Oral	50	60	48	6.4	32.3	90	30	23	4.9
	25	Oral	50	60	85	6.5	48.5	100	60	72[b]	8.3
	50	Oral	30[a]	10	50	12.9	35.7	100	60	37	6.2
	50	Oral	50	60	92	7.0	70.6	100	60	7	6.0
	50	Oral	..	..	..	..	..	140[a]	10	50	4.4
	10	Vein	30[a]	20	65	1.2	4.7	20[a]	20	55	0.0
	25	Vein	30	10	100	0.0	22.7	..	..	..	..
Glycerin	10	Oral	50	60	38	6.3	27.6	100	60	52[b]	6.6
	25	Oral	50	60	5	8.8	18.3	100	60	37	10.7
	50	Oral	50	60	3	18.2	12.5	100	60	25[b]	29.0
	50	Oral	100[a]	10	50	5.2	28.0	125[a]	10	50[b]	12.6
	10	Vein	27.5[a]	10	50	0.6	4.1	20[a]	20	55	0.0
	10	Vein	30	20	70	1.4	4.8	20	10	80[b]	0.0
	25	Vein	30	10	100[b]	0.0	19.7	..	..	..	..
Alcohol	10	Oral	30[a]	10	60	3.1	25.6	150[a]	10	50[b]	3.2
	10	Oral	50	60	97	3.9	62.6	100	60	17	24.5
	25	Oral	50	60	100[b]	4.6	1534.0	100	60	0	..
	50	Oral	50	60	100	3.5	1013.0	100	60	0[b]	..
	10	Vein	30	20	100	0.0	17.5	20	20	0	..
	10	Vein	..	..	..	..	..	60[a]	10	40	0.0

[a] Approximate ED_{50} under the conditions tabulated.
[b] Mortality in excess of 10%.

either drug (Table II) follows a pattern analogous to that discussed for propylene glycol, but the interaction is milder while the systemic action of glycerin tends to be more pronounced.

In view of the marked effect which the higher concentrations of glycerin exerted on the orally administered drugs, a separate series of experiments was set up to determine the duration of this effect. Several groups of 10 mice each were given the standardized volume of 50% glycerin orally and at half-hour periods following the glycerin 30 mg./Kg. of sodium pentobarbital was administered orally. Only an occasional mouse lost its righting reflex even three and a half hours after the administration of the glycerin. When the experiment was repeated with 25% glycerin the sodium pentobarbital was effective when administered one-half hour after the glycerin. From these results it appears that the degree and duration of interference with absorption produced by glycerin are proportional to the concentration present and this agrees well with the results discussed above.

Alcohol.—Reference to Table I shows that the synergism between alcohol and sodium pentobarbital and the antagonism between alcohol and metrazol completely overshadow any change that there may be in absorption. Following oral administration of the drugs in 10% alcohol this interaction amounts to 40% for sodium pentobarbital and 50% for metrazol. Since intravenous administration of 10% alcohol produces loss of righting reflex in 50% of the animals, it is impossible to evaluate the intravenous interaction between alcohol and sodium pentobarbital, when administered simultaneously, but with metrazol it is approximately 200%. When the alcoholic vehicle and the drugs are separated for oral and intravenous administration (Table II) the interaction follows the usual pattern if the alcohol is administered orally. When, however, the alcohol is administered intravenously a definite

interference with the action of both sodium pentobarbital and metrazol is observable. In the case of the sodium pentobarbital the incidence of loss of righting reflex approximates that of the same dose administered orally in the control series. This is the only time in any of the experiments that the combination of sodium pentobarbital and alcohol in appropriate dosage failed to produce almost 100% effect. It is to be remembered that intravenous injection of standardized dose of 10% alcohol causes loss of righting reflex in approximately 50% of the animals. This effect is transient, however, and the animals regained the reflex before the orally administered sodium pentobarbital became effective. This undoubtedly accounts for the quicker onset of the barbiturate effect but does not increase the percentage. Because of the marked antagonism between alcohol and metrazol the 150 mg./Kg. dose of metrazol was given orally following injection of 10% alcohol. While there is a slight increase in the percentage of responses in this series compared with the same dose administered orally in 10% alcohol, it is significant to note that the time of onset is very markedly delayed. Under these conditions it appears, therefore, that 10% alcohol is capable of a systemic action which interferes with the absorption of sodium pentobarbital and metrazol, but that under the usual conditions of oral administration this effect is not apparent.

Interaction of the Vehicle Ingredients.—Many pharmaceutical elixirs contain both sucrose and alcohol. In experiments previously described it was noted that 50% sucrose markedly reduced the activity of sodium pentobarbital, while 10% alcohol increased the action. Interpretation of the results makes it apparent that the sucrose acts by interference with absorption while the alcohol acts by pharmacological synergism. The interesting question therefore arises as to whether these two ingredients, acting by two different mechanisms, are in themselves antagonistic. To examine this question the ED_{50} of sodium pentobarbital was administered orally in a vehicle containing 50% sucrose plus 10% alcohol, and 35% of 20 mice showed loss of righting reflex compared with 10% with this dose in 50% sucrose and 97% with this dose in 10% alcohol. The experiment was repeated with a vehicle containing 30% sucrose plus 20% alcohol and 95% of 20 mice showed loss of righting reflex. In both experiments the time of onset and the duration of effect were proportional to the percentage of incidence. It is thus apparent that the interference with absorption by sucrose may be antagonized by the synergism with alcohol and vice versa.

TABLE II—RESULTS FOLLOWING SEPARATE ADMINISTRATION OF DRUG AND VEHICLE, ONE INTRAVENOUSLY AND THE OTHER ORALLY[a]

Route of Administration		Dose of Drug, Mg./Kg.	No. of Mice	End Point, %	Onset, Min.	Duration, Min.
Intravenous	Oral					
Sodium pentobarbital	Sucrose, 50%	30	10	30	1.6	4.8
Sucrose, 10%	Sodium pentobarbital	50	10	50	3.3	39.0
Metrazol	Sucrose, 50%	20	10	60	0.0	..
Sucrose, 10%	Metrazol	100	10	40	3.6	..
Sodium pentobarbital	Dextrose, 50%	30	10	60	3.9	10.5
Dextrose, 10%	Sodium pentobarbital	50	10	40	8.0	9.5
Metrazol	Dextrose, 50%	20	20	50	0.0	..
Dextrose, 10%	Metrazol	100	10	60	3.1	..
Sodium pentobarbital	Propylene Glycol, 50%	30	15	93	2.5	21.0
Propylene glycol, 10%	Sodium pentobarbital	50	10	30	11.6	3.8
Metrazol	Propylene glycol, 50%	20	10	0	..	..
Propylene glycol, 10%	Metrazol	100	10	30	7.3	..
Sodium pentobarbital	Glycerin, 50%	30	10	80	1.8	6.8
Glycerin, 10%	Sodium pentobarbital	50	10	10	8.0	5.0
Metrazol	Glycerin, 50%	20	10	40	0.0	..
Glycerin, 10%	Metrazol	100	10	20	3.7	..
Sodium pentobarbital	Alcohol, 10%	30	10	100	0.8	29.0
Alcohol, 10%	Sodium pentobarbital	50	10	60	3.8	28.0
Metrazol	Alcohol, 10%	20	10	10	0.0	..
Alcohol, 10%	Metrazol	150	10	70	10.3	..

[a] See text for details.

DISCUSSION

An over-all inspection of the above results indicates that four principal types of interaction between drug and vehicle were encountered:

1. Interference with Absorption of the Drug. This phenomenon is demonstrated by glycerin, sucrose, and dextrose administered orally and is presented schematically in Fig. 1. Impaired absorption is the causative mechanism because oral administration of the drug in the specified vehicle produces a marked decrease in the response to *both* drugs. Intravenous administration of either drug in the vehicle shows no signifi-

cant pharmacological interaction, nor does administration of the vehicle orally and the drug intravenously. It is probable that this type of action would not be limited to the drugs studied.

Evidence is cited to indicate that glycerin, propylene glycol, and alcohol when administered intravenously tend to interfere with gastrointestinal absorption but that in general this action is overshadowed by other types of interaction. Sucrose and dextrose do not appear to exhibit this systemic action.

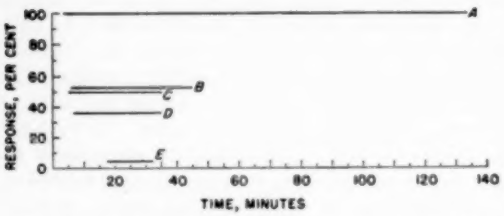

Fig. 1.—Action pattern of sodium pentobarbital administered orally in a glycerin-containing vehicle. Solid line represents percentage of response, time of onset, and duration of action.

Key: *A* = 100 mg./Kg. sodium pentobarbital in water
B = 50 mg./Kg. sodium pentobarbital in water
C = 100 mg./Kg. sodium pentobarbital in 50% glycerin
D = 50 mg./Kg. sodium pentobarbital in 10% glycerin
E = 50 mg./Kg. sodium pentobarbital in 50% glycerin

2. Pharmacological Synergism Between the Drug and Vehicle. This phenomenon is demonstrated by propylene glycol or alcohol with sodium pentobarbital and is presented schematically in Fig. 2. Evidence for this mechanism of action is as follows: oral administration of the drug in the vehicle exceeds in both incidence and duration of action the results following the aqueous standard, and intravenous administration of the drug in the vehicle follows the same pattern. The separate administration of the drug and vehicle by oral and intravenous routes results in synergism when the vehicle is given orally, but when the vehicle is given intravenously the systemic action may be evident as mentioned in the preceding section. The degree of interaction following oral administration of the drug and vehicle may be evaluated either by increasing the dose of the drug in water or decreasing its dose in the vehicle. This is also illustrated in Fig. 2.

3. Pharmacological Antagonism Between the Drug and Vehicle. This is demonstrated by propylene glycol or alcohol with metrazol, and is presented schematically in Fig. 3, in which duration is estimated rather than observed (11). Criteria for this type of interaction are reduced oral potency of the drug in the vehicle, combined with a similar decrease following intravenous administration and when the vehicle is given orally and the drug intravenously. It is again to be noted that alcohol, glycerin, or propylene glycol intravenously alters the action of sodium pentobarbital or metrazol given orally. The degree of interaction may be evaluated by increasing the drug dosage in the vehicle. This result may be compared with the dose required for comparable effect in water or with the results obtained with the increased dose in water. The principles of bioassay should be observed when making such comparisons.

4. Interaction Between Two Vehicle Ingredients. This type of interaction, which occurs in typical elixirs, is demonstrated by a mixture of sucrose which interferes with absorption and alcohol which synergizes with

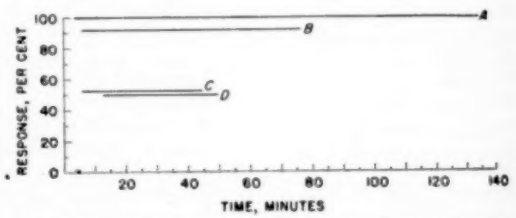

Fig. 2.—Action pattern of sodium pentobarbital administered orally in vehicle containing propylene glycol. Solid line represents percentage of response, time of onset, and duration of action.

Key: *A* = 100 mg./Kg. sodium pentobarbital in water
B = 50 mg./Kg. sodium pentobarbital in 50% propylene glycol
C = 50 mg./Kg. sodium pentobarbital in water
D = 30 mg./Kg. sodium pentobaribital in 50% propylene glycol

sodium pentobarbital and is presented schematically in Fig. 4. Inspection of this figure indicates that this phenomenon indeed behaves like a true mutual antagonism. This type of antagonism appears to have been encountered by Dille and Seeberg (1) in

their study on barbital elixir N. F. VII wherein it is pointed out that the barbital was poorly absorbed due to the glycerin, but that the degree of depression was greater than would be expected from the barbiturate blood level. This was attributed to synergism between alcohol and the barbiturate. Seeberg (2) in effect substituted the interfering action of sucrose for that of a high concentration of glycerin and retained the synergistic action of approximately equal quantities of alcohol. While the barbiturate absorption is improved in the proposed elixir, the blood barbiturate level is still insufficient to account for the degree of depression and the synergism with alcohol is thus apparent.

Figures 1, 2, 3, and 4 present the grossly observed picture following oral administration of sodium pentobarbital or metrazol in the vehicle noted. The striking similarity between Figs. 1 and 3 illustrate the difficulty of evaluating the individual factors by observation of the orally administered combinations only. If, however, these results are correlated with the results following intravenous administration and following administration of the drug by one route and the vehicle by another, the type of influencing factor becomes apparent. Further interpretation of the data makes it possible not only to determine the dominant factor involved in the change of action but to detect less prominent factors.

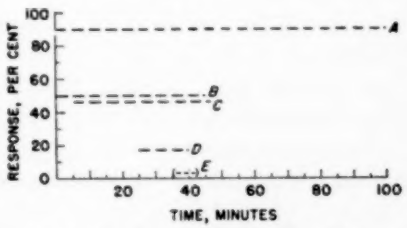

Fig. 3.—Action pattern of metrazol in a vehicle containing ethyl alcohol. Broken line represents per cent of response, time of onset, and estimated duration of action.

Key: *A* = 150 mg./Kg. metrazol in water
B = 150 mg./Kg. metrazol in 10% alcohol
C = 100 mg./Kg. metrazol in water
D = 100 mg./Kg. metrazol in 10% alcohol
E = 100 mg./Kg. metrazol in 25% alcohol; gives no response

In the course of presenting the results of these experiments and their brief discussion many observations of interest to the investigators have been omitted. It has been tempting to try to elucidate the fundamental mechanism of interference with absorption by correlation with the known biochemical action of such drugs as sucrose, dextrose, metrazol, and sodium pentobarbital, particularly their effect on blood-sugar levels.

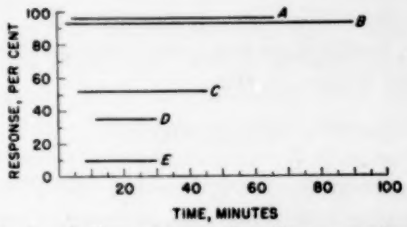

Fig. 4.—Action pattern of sodium pentobarbital administered orally in a vehicle containing a combination of sucrose and alcohol. Solid line represents percentage of response, time of onset, and duration of action.

Key: *A* = 50 mg./Kg. sodium pentobarbital in 10% alcohol
B = 50 mg./Kg. sodium pentobarbital in 30% sucrose plus 20% alcohol
C = 50 mg./Kg. sodium pentobarbital in water
D = 50 mg./Kg. in 50% sucrose plus 10% alcohol
E = 50 mg./Kg. in 50% sucrose

The correlation of chemical structure with the pharmacological interaction, systemic action and effect on absorption of alcohol, propylene glycol, and glycerin makes interesting speculation. The interference with barbiturate action is consistently more pronounced than the interference with metrazol action, but these problems and others of this type are beyond the scope of the present paper. The onset time for sodium pentobarbital and metrazol and the duration of action for sodium pentobarbital have been presented completely in the tables but only an occasional reference to them has been made in the text of the paper. Careful study of these values will reveal some interesting results which suggest an influence other than those presented herein. In general, however, these two factors correspond closely enough to the dose effect curves which might be expected, when incidence of action is taken as an index, that using either onset time or duration for sodium pentobarbital or onset time for metrazol would not appreciably change the conclusions which have been drawn.

Throughout these experiments it appears that interaction of the drug and vehicle is influenced by the concentration of the vehicle ingredient rather than the absolute amount present. On the other hand it has been pointed out that the drug dosage used was far in excess of the therapeutic dose. It therefore becomes difficult, and perhaps inadvisable, to draw direct clinical implications from these experimental results. Ultimate verification can come only from controlled clinical studies.

CONCLUSIONS

1. Sucrose, dextrose, and glycerin in concentrations above 10 per cent interfere with the gastrointestinal absorption of sodium pentobarbital or metrazol in mice following oral administration. Propylene glycol and alcohol do not. This appears to be a local phenomenon distinct from systemic action.

2. Oral administration of alcohol in concentrations of 10 per cent or more increases the action of sodium pentobarbital and decreases the action of metrazol. This is a systemic action independent of any local effect.

3. Propylene glycol exerts an action qualitatively similar to alcohol but quantitatively less.

4. Evidence is presented to indicate that glycerin, alcohol, and propylene glycol, following intravenous administration, exert systemic actions which interfere with the absorption of both sodium pentobarbital and metrazol, but that following oral administration these actions are not apparent.

5. Two vehicle ingredients, as exemplified by a solution containing both sucrose and alcohol, may be mutually antagonistic in their influence on drug action even though their individual mechanism of action is different. The degree of antagonism is a factor of the relative concentration.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

N-(3,4-Dihydroxyphenyl)-glycine
Glucose-1,6-diphosphate
Lycorine and derivatives
1-3,4-Dihydroxyphenylalanine
Arachidonic acid
2,4-Dinitrofluorobenzene
Myricitrin
Eriodictyol
Tectoridan
Homoeriodictyol
Myricetin
Rhamnetin
4,5-Di-(4-hydroxyphenyl)octane
1-Methyl-2,3-diethyl-2,3-di(4-hydroxyphenyl)-propane
1-Ethyl-2-(4-hydroxyphenyl)-5-hydroxyindene
Stilboestrol mono glucoside
4,4′-Dihydroxy-α-methyl-β-propylstilbene
4,4′-Dihydroxy-α-methyl-β-isopropylstilbene
Gossypetin
Cannabiscetin

The Effect of Continued Oral Administration of Lauric and Myristic Acid Ester of Colaminoformylmethylpyridinium Chloride (Emulsept) to the Albino Rat*

By A. EARL VIVINO and THEODORE KOPPANYI

AN EFFICIENT, safe, and odorless antiseptic and germicidal concentrate for controlling bacteria count and for sterilizing food-processing equipment as well as the hands of food-handling personnel is a desideratum from a public health standpoint. The recently developed detergent agents, particularly the cationic detergents, appear to fulfill these requirements better than some of the older antiseptics.

The efficacy of the lauric and myristic acid ester of colaminoformylmethylpyridinium chloride (Emulsept[1]) as an antiseptic has been reported in several publications (1–4). This quaternary ammonium derivative is characterized by the presence of an ester linkage in the cationic part of the molecule.

The relative toxicity index of "Emulsept" has been determined by Welch and Brewer (5, 6) by comparing the destruction of the phagocytic activity in the blood (A) (essential tissue toxicity) with its toxicity for *Staphyloccus aureus* (B) (germicidal capacity). A low toxicity index (A/B) in such tests would indicate wider margin of safety (toxicity index for Emulsept 0.5 *vs.* chloramine T 1.16; crystal violet 1.42; formaldehyde 30). Cationic detergents as a group, and Emulsept in particular, were found to exhibit marked bactericidal effects on Gram-positive organisms and somewhat less pronounced effect on Gram-negative organisms according to Baker, Harrison, and Miller (7).

Hirsch and Novak (8), also using the above tissue toxicity index, confirmed these results, and concluded that by this method the organic mercurials rate the lowest as efficient germicides. They believe that the phagocyte inhibition technique may be regarded as a valuable aid in the evaluation of germicides for clinical use. If that be correct, this test is probably even more valuable as an index of safety of these compounds when used as cleaning agents.

Finally, Whitehill (9) determined the nonirritating concentrations of various antiseptics using ophthalmic changes as a criterion. In rating the degree of ocular injury produced by the antiseptic upon instillation, photophobia was taken as the mildest and opacity of the cornea as the most severe degree of injury. The irritant effects were then compared with the germicidal power. Judging by these standards he found that cationic substances, including "Emulsept," make the "best active" antiseptics while inorganic mercury compounds were the poorest, when rated according to the time it required for their maximum nonirritating concentrations to kill *Staphylococcus aureus*.

The maximum dose of "Emulsept" tolerated by the rat after intraperitoneal injection of a 10 per cent solution is 125 mg. per kg. body weight (10).

The margin of safety of the cationic agents in general, and that of "Emulsept" in particular, has been amply established in acute experiments. Since, however, these cationic agents were recommended and have, in fact, been used as sterilizing rinses or sprays in many different fields it appears that the study of the chronic toxicity of this compound is at least as important as the various toxicity indices determined in acute experiments. The purpose of this paper is to report on the effect of continued oral administration of this new cationic detergent in rats.

* Received Dec. 14, 1945, from the Dept. of Pharmacology and Materia Medica, Georgetown University School of Medicine, Washington, D. C.

[1] Emulsept ($[R-\overset{O}{\overset{\|}{C}}-O-C_2H_4NH\overset{O}{\overset{\|}{C}}-CH_2-N\langle\rangle]^+ Cl^-$) obtained in 10% solution from The Emulsol Corporation, Chicago 3, Ill.

EXPERIMENTAL

Procedure.—Albino rats of the Sprague–Dawley strain representing three generations were used in this investigation. Daily observations were made on all rats relative to their appearance and activity, appearance and consistency of the feces, and color and odor of the urine. Food and water were given daily and all cages, bottles, and food containers were cleaned weekly. The rats were weighed weekly on an accurate metric balance to obtain the growth rates. The rats of the parent (P) generation were shipped from Wisconsin by Railway Express and were received when thirty days of age. These rats were placed in large ventilated cages, not more than five to a cage, for a period of two weeks to become adjusted to the environment and the basic diet of Purena Layena. This diet contained Carotene (Pur-A-Tene), dried milk by-product, riboflavin supplement (Puri-Flave), vitamin A and D feeding oil, meet scrap, ground oats, fish meal, soybean oil meal (Expellor Process), alfalfa meal, wheat middlings (gray), dried beet pulp, corn meal, 0.5% iodized salt, and 4% calcium carbonate. The guaranteed analysis was 15.5% protein, 3.5% fat, 8% fiber, and 47% nitrogen-free extract. After the period of adjustment the rats were put on a diet containing "Emulsept." The control group of the P generation, consisting of 26 rats (11 males and 15 females), remained on the Purena-Layena diet for twenty-five weeks, tap water being given as the source of fluid. The experimental group of the P generation consisting of 25 rats (16 males and 9 females) received the same diet of Purena-Layena, but their only source of fluid was a 0.1% solution of the active ingredient of "Emulsept." The 0.1% solution was placed in water bottles specially designed to prevent evaporation and mixing of water with excreta. These rats remained on this diet of Purena-Layena and "Emulsept" solution for twenty-five weeks.

The rats of the P generation were inbred as soon as they reached maturity to form the F1 generation. The latter group was in turn inbred to form the F2 generation. In each case males were mated to females in their respective groups with no change in their respective scheduled treatment. After weaning, the groups were placed in cages similar to that described above and continued on the same specific regimen as the parents. The F1 generation from the control group, numbering 30 rats (12 males and 18 females), was continued on the diet of Purena-Layena. The F1 generation from the experimental group, numbering 14 rats (7 males and 7 females) continued to receive the diet plus a 0.1% solution of active ingredient of "Emulsept." All the rats of the group remained on the diet for twelve weeks after weaning. The parent rats did not have any change of diet during either the periods of gestation or lactation. The experiments on the third generation (F2) were limited to 6 males in each group because of lack of space. The diets for the experimental and control groups continued the same as for the parents for four weeks at which time the experiment was terminated.

At the conclusion of the experiment the spinal cord was pithed to render the animal insensitive to pain and then the rats were quickly decapitated. Careful necropsy was performed on each rat. The liver, stomach, and kidneys were removed from two rats from each group of the treated and untreated rats in the P, F1, and F2 generations. These tissues were fixed with Bouin's Solution, then prepared for microscopic study. The tissues were embedded in paraffin, sectioned at 10 microns, stained with Ehrlich's acid, hematoxylin counterstained with eosin.

RESULTS

The amount of food eaten by the rats ranged between 7 and 15 Gm. daily per rat with an average of 10 Gm. per rat per day. The volume of liquid ingested by the rats averaged 20 ml. per rat per day. Therefore, the ingestion of the active ingredient of "Emulsept" was 20 mg. per day. The effect of the synthetic detergent on the growth of albino rats is summarized in Table I. This table shows the number of animals in each group, the duration of the experiment, and the average weekly weight increase per rat. It is observed that experimental rats of the first, second, and third generations show as much average weekly weight increase as the control animals. The male rats of the P generation show an average weekly weight increase for twenty-five weeks of 8.0 and 8.4 Gm. (see Table I) for the control rats and for rats on "Emulsept," respectively. Male and female treated rats of the P generation during comparable weighing periods of four weeks show little variation in body growth increase from the control, although there is a greater weight increase in the males than in the females in this generation. There is a tendency for males to outgrow the females even in animals on control diet.

Over a period of twelve weeks the male rats of the F1 generation show an average weekly weight increase of 9.4 and 13.8 Gm. (see Table I) for the control and rats on "Emulsept," respectively. Males and females of treated rats of the F1 generation on comparable weighing periods of seven weeks show little variation from the control, although again there is a greater increase in body weight among the males than among the females in this generation.

In four weeks the male rats of the F2 generation show an average weekly weight increase of 18.5 and 17.6 Gm. for the control rats and for rats on "Emulsept," respectively.

The curves plotted on the graphs (Figs. 1–5) represent the average weekly increase in body weight of the rats in the first, second, and third generations. These growth curves indicate that the body weight increase of treated animals as compared with that

TABLE I—EFFECT OF "EMULSEPT" INGESTION ON AVERAGE WEEKLY WEIGHT INCREASE OF ALBINO RATS

No. of Animals			Duration of Experiment, Weeks		Average Weekly Weight Increase		
Males	Females		Males	Females[a]	Males	Males	Females
					25-Wk. Period	4-Wk. Period	
		Parent Generation (P)					
11	15	Control	25	4	8.0	24.2	12.5
16	9	"Emulsept"	25	4	8.4	26.2	15.0
		First Generation (F1)					
					12-Wk. Period	7-Wk. Period	
12	18	Control	12	7	9.4	15.0	13.0
7	7	"Emulsept"	12	7	13.8	23.1	14.3
		Second Generation (F2)					
					4-Wk. Period	4-Wk. Period	
6	..	Control	4	..	18.5	18.5	..
6	..	"Emulsept"	4	..	17.6	17.6	..

[a] Used for breeding; necessitated cessation of weighing of females because of pregnancy, although the feeding of the respective diets was continued until the end of the experiment.

of the untreated animals follows the same general trend and is similar. In these experiments it was necessary to record separately the data on growth of the males and females, and thus to represent graphically their rate of growth on separate charts. From these charts it is observed that the general weight increase of the females of the treated as compared with the untreated (see Figs. 2 and 4) is similar and that the rate of growth of the females is less than the growth increase of the males. A comparison of the body growth of the treated males

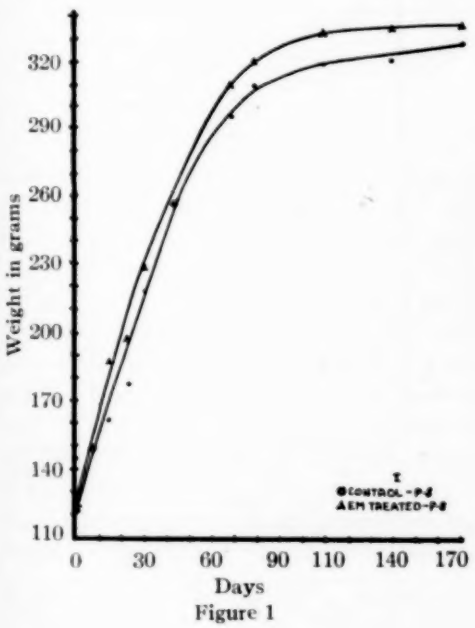

Figure 1

with the untreated males shows similarity in general growth trend (see Figs. 1, 2, and 5).

It is apparent that the ingestion of this cationic synthetic detergent had little inflenuce on the general

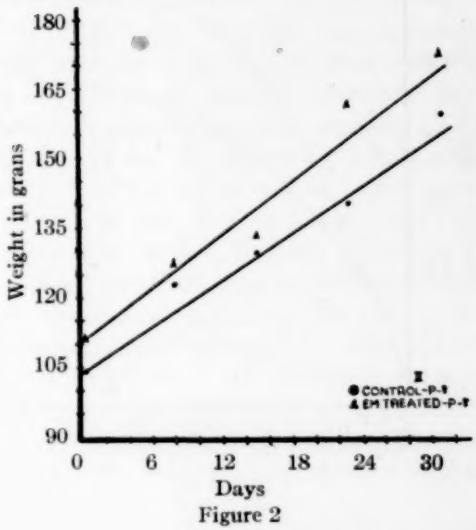

Figure 2

body weight increase of both male and female albino rats when compared to the control untreated animals, and that the growth rates in all were within the normal range. This fact indicates that the rats had a normal appetite and did not become anorexic, and showed no manifestation of malnutrition on the diet containing the detergents.

This indicates further that the oral administration of the synthetic detergent did not interfere with the usual passage of vitamins, minerals, and essential foods through the gastrointestinal tract mucosa to be utilized by the body.

Throughout these experiments the feces were solid, well formed and of similar appearance and color in all cases. No diarrhea was ever produced in any of the rats and no rat showed nausea. These facts indicate the lack of gastrointestinal irritation from the ingestion of the synthetic detergents.

The rats showed no signs of hyperirritability, depression, or muscular weakness, indicating nor-

mal function of central and peripheral nervous systems.

The females gave birth in all cases to an average litter of rats. These litters were well cared for by the mothers and grew in the normal manner. None of these rats died either at birth or before the termination of the experiment. The texture of the fur appeared normal in all cases and the skin, mucous membrane, and eyes were apparently free from pathological changes. The lack of any such disturbance indicates proper nutrition of these tissues and that the synthetic detergent tested caused no disturbance of metabolism in the rats. A gross examination of the liver, kidney, and gastrointestinal tract did not reveal any changes from normal controls, indication of an absence of chronic gastritis or enteritis.

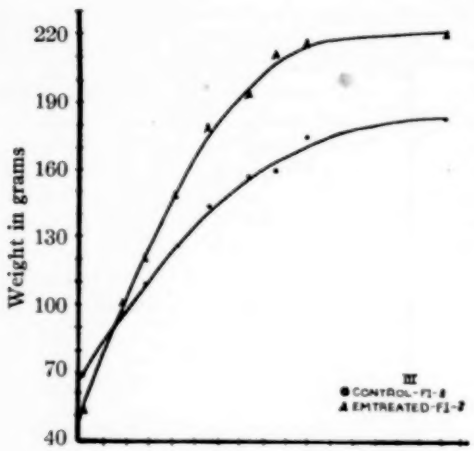

Figure 3

The microsopical appearance of the liver, kidney, and stomach appeared normal in all three generations of both treated and untreated animals, except in the kidney of one experimental animal in the F1 generation which had a mild amyloidosis of the proximal tubule epithelium. This pathology was not observed on the kidneys of other animals undergoing the same treatment and may not be significant.

DISCUSSION

In the sterilization of dishes, glasses, utensils, and food-processing equipment, both physical and chemical methods have been employed. According to Rahn (11), physical means of destruction of bacteria include such methods as mechanical (pressure changes and ultra-sonic waves), irradiation (wave length and temperature effects), desiccation, low temperatures, and moist heat. Another very important method of sterilization is the use of surface-tension depressants. Many compounds such as fatty foods, dyes, alcohols, bile salts, and wetting agents (all of which lower the surface tension of water) are disinfectants. Such wetting agents as soap have been in use for many centuries, but are gradually being replaced by the newer synthetic detergents which have a greater effect on surface tension. It is generally known that some synthetic detergents are efficient in acid as well as in alkaline media and are not precipitated by hard water. They are also effective in increasing the efficiency of other antiseptics by augmenting their rate of diffusion through the cell membrane.

Thus, the effects of chronic oral administration of these compounds become of practical importance from the standpoint of public health considerations. The results obtained in our investigation indicate that

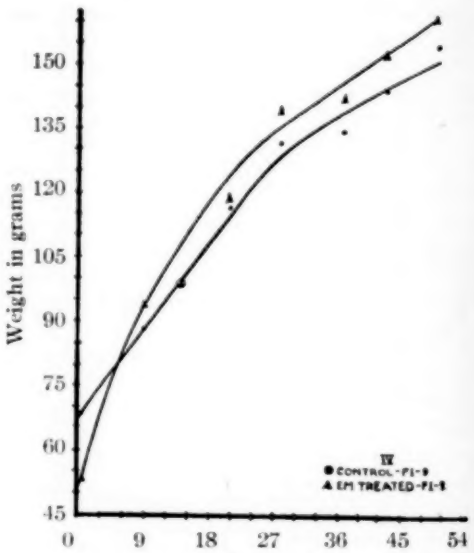

Figure 4

"Emulsept," a cationic detergent, when administered by mouth in concentrations even higher than is likely to occur by accidental drinking of moderate amounts of cleansing solutions used in handling of food and processing equipment does not produce

deleterious effects in rats even with prolonged administration carried through several generations. In fact, the rats in our investigation received their total liquid intake in the form of a 0.1 per cent solution of "Emulsept," whereas "Emulsept" is being used as a sterilizing rinse in food processing in 0.04 per cent solution. Assuming, therefore, that human beings are no more affected than rats by the detergent, no harmful effects could result from using this solution to quench thirst much less from traces of such solutions that happen to adhere to dishes or food particles.

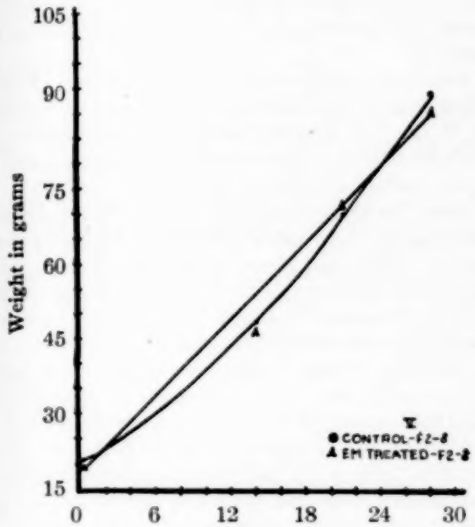

Figure 5

Tepley and Elvehjem (12) made a similar study on the chronic toxicity of a germicidal quaternary ammonium compound ("Phemerol") using the Sprague–Dawley strain of rats. They found that when "Phemerol" was incorporated in the solid synthetic diet at the level as low as 0.1 per cent the rats refused to eat the food and soon died from inanition. This diet mixture was found to be irritating to the throat. Young rats did not survive a 1:1000 solution of "Phemerol," no matter what was added to the synthetic diet. A comparison between these results and the results described above shows that "Emulsept" is nonirritating and is readily accepted in the drinking water.

SUMMARY AND CONCLUSION

"Emulsept," a cationic synthetic detergent which is widely used as an antiseptic for the sterilization of food-processing equipment and the hands of food-handling personnel, shows no evidence of toxicity in the amounts administered orally to Albino rats for three generations. The facts warranting this conclusion may be summarized as follows:

1. The average weekly increase in body weight on the basis of the gross weights and growth curves of the treated rats and those of the untreated rats was comparable.

2. The conclusion that the appearance and behavior of the rats on diets containing a mixture of the synthetic detergent and a well-balanced ration were normal, may be supported by the following observations: normal eating habits and appetite, normal texture of fur, skin, mucous membranes, eyes, reproductive activity and muscular activity, and lack of anorexia, malnutrition, diarrhea, and nausea. These normal physiological functions indicate that the oral administration of the synthetic detergent did not interfere with the usual passage of vitamins, minerals, and essential foods through the gastrointestinal tract mucosae to be utilized in the body.

3. No significant pathological alterations were observed upon microscopic examination of the gastrointestinal tract, liver, lungs, kidneys, and bladder.

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Determination of Zinc Oxide and Bentonite in Calamine Lotion*

By CHARLES O. WILSON† and HENRY G. SPERLING‡

A procedure for determining the total zinc oxide in Calamine Lotion, N. F. VII is presented, and a method indicating the quantity of bentonite and ferric oxide in Calamine Lotion, N. F. VII is suggested.

CALAMINE Lotion first appeared as an official preparation in the National Formulary V. The National Formulary VII added bentonite to the formula. At the present time there exists no published procedure for the determination of zinc oxide and bentonite in Calamine Lotion. The procedure outlined in this paper for the determination of zinc oxide was developed to make possible the standardization of Calamine Lotion.

Since 1942, the State Board of Pharmacy of the State of Minnesota has supported an analytical laboratory for the analysis of drug products. Calamine Lotion is prepared by many pharmacies, and upon examination of over one hundred samples, many variations were observed. The determination of zinc oxide and bentonite in Calamine Lotion provides a means of insuring a uniform lotion.

EXPERIMENTAL

Preparation of Calamine Lotion, N. F. VII.—The Calamine Lotion was prepared according to National Formulary VII. Exactly 1000 cc. was prepared in a tared glass-stoppered, graduated container. Two lotions, A and B, were prepared, each of them having a volume of one liter and weighing 1092.4 Gm.

Determination of Zinc.—Zinc ions in neutral or acid medium react with potassium ferrocyanide to form very slightly soluble potassium zinc ferrocyanide.

$$2K_4Fe(CN)_6 + 3Zn^{++} = K_2Zn_3[Fe(CN)_6]_2 + 6K^+$$

This reaction has been used for more than sixty years for the determination of zinc. Until recently, the end point was detected by means of external indicators, but it has been found that diphenylamine is suitable as an internal indicator (1).

Procedure for Assay

Reagents.—Standard zinc, 0.1 *M* solution: Dissolve 6.538 Gm. of pure reagent quality zinc in 10% sulfuric acid to make 1000 cc. The exact molarity can be calculated from the quantity of zinc used.

Potassium ferrocyanide, 0.05 *M* solution: A reagent quality $K_4Fe(CN)_6{\cdot}3H_2O$ is used. Dissolve 21.12 Gm. of $K_4Fe(CN)_6{\cdot}3H_2O$ in distilled water to make 1000 cc. The exact molarity can be calculated from the quantity of salt used.

Potassium ferricyanide solution: A 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution in distilled water is used. This must be stored in light-resistant containers and prepared fresh every three or four days.

Diphenylamine indicator: A 1% diphenylamine in concentrated sulfuric acid is used.

Sampling.—Shake Calamine Lotion well and obtain a cross section of the sample by inserting a glass tube of suitable length (having about an 8-mm. inside diameter) to the bottom of the container. Allow glass tube to fill, place finger tightly over open end, quickly withdraw the tube, and drain into a tared 250-cc. Erlenmeyer flask.

Procedure.—Place about 10 Gm. of Calamine Lotion in a tared 250-cc. Erlenmeyer flask, weigh and add 100 cc. of approximately 2.0 *N* sulfuric acid. Warm on steam bath until zinc oxide and calamine have dissolved, leaving only bentonite and ferric oxide. This requires about fifteen minutes. Transfer all of the material to a 150-cc. centrifuge tube and centrifuge three to five minutes and decant supernatant liquid through quantitative filter paper into a 500-cc. volumetric flask. To residue in centrifuge tube, add 50 cc. distilled water, mix well, centrifuge, and decant through filter paper as before. Repeat this twice. Transfer precipitate completely to filter paper and wash once with 10 cc. of distilled water. Place filter paper containing residue in tared porcelain crucible and ignite to constant weight for the determination of bentonite and ferric oxide (see Table II).

Make filtrate up to exactly 500 cc. and pipette a 50-cc. sample into a 250-cc. Erlenmeyer flask; add 75 cc. distilled water, 2.0 Gm. of ammonium sulfate, 3 drops of potassium ferricyanide solution, and 3 drops of diphenylamine indicator. Wait until the blue color develops, and titrate past the end point with 0.05 *M* potassium ferrocyanide solution until the blue color changes to a light, creamy, yellowish green, then continue titration slowly to the nearest even centimeter. Back titrate slowly with 0.1 *M*

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standard zinc solution to the appearance of the purple color. A sharp color change is produced at the end point.

Notes.—(1) The appearance of the initial blue color is somewhat facilitated if a few milliliters of ferrocyanide solution are added before the addition of the ferricyanide and indicator. (2) In order to obtain accurate results (within 0.3%) the conditions under which the titration is carried out must be rigidly controlled and should be exactly the same as in the standardization. The speed of titration near the end point and the excess of ferrocyanide added before the back-titration with zinc must be kept uniform. The inaccuracy increases with the amount of ferrocyanide added in excess.

Calculations

$$\frac{\text{cc. of } 0.05\ M(K_4Fe(CN)_6)(0.0061) - (\text{cc. } 0.1\ M\ Zn)(0.008138)\ 10}{\text{Wt. of Calamine Lotion Sample}} \times 100 = \%ZnO$$

0.0061 = Grams of ZnO per cc. of 0.05 M $K_4Fe(CN)_6$
0.008138 = Grams of ZnO per cc. of 0.1 M Zinc

The results of analyses are given in Table I.

TABLE I

Lotion	Sample	Calculated, % w/w ZnO	Found, % w/w ZnO
A	1	14.46	14.32
A	2	14.46	14.31
B	3	14.46	14.46
B	4	14.46	14.45

Purity of Ingredients

Zinc Oxide, U. S. P. XII was heated on a Meker burner for five hours to constant weight; the loss was 0.37%. The sample was assayed by the given method, using a 4.0-Gm. sample and the U. S. P. XII procedure. In each case, the zinc oxide was found to be 99.3%.

Calamine was heated on a Meker burner for five hours to constant weight, the loss being 0.27%. The calamine was assayed by the given method using a 4.0-Gm. sample and the National Formulary VII procedure.

Given Method: 98.68% and 98.82%, av. 98.75%
N. F. VII Procedure: 98.72% and 98.80%, av. 98.76%

Bentonite, U. S. P. XII was heated on a Meker burner for five hours to constant weight; the loss was 11.63%.

Determination of Bentonite and Ferric Oxide.—The removal of the insoluble portion by filtration after centrifugation is described in the preceding procedure. This residue consists of bentonite and ferric oxide and is collected, ignited and weighed. Calculations show that each gram of Calamine Lotion should contain 18.1 mg. of bentonite and not more than 1.4 mg. of ferric oxide.[1] Since bentonite loses 11.63% on heating, the weight after ignition is (18.1 × 88.37%) 16.0 mg. The weight of residue collected on filter paper during the assay for zinc oxide in Calamine Lotion after ignition should be about (16.0 + 1.4) 17.4 mg. per Gm. of sample.

The results of analyses are given in Table II. Since 1.4 mg. of ferric oxide is the maximum quantity that may be present in one gram of Calamine Lotion, it is reasonable to assume that many lotions will contain less than this amount. Upon this assumption, the results expressed for Gm. of Bentonite-Ferric oxide found per Gm. of sample seem very satisfactory.

This approach serves both as a qualitative and a quantitative index of bentonite in Calamine Lotion, little or no residue on the filter paper indicating the absence of bentonite.

TABLE II

Lotion	Wt. of Sample in Gm.	Theoretical Wt. in Gm., Bentonite-Ferric Oxide	Gm. Found of Bentonite-Ferric Oxide	Gm. of Bentonite-Ferric Oxide. Found per Gm. of Sample
A	9.4470	0.1644	0.1547	0.0163
A	8.9838	0.1561	0.1470	0.0163
B	8.7428	0.1552	0.1448	0.0165
B	13.1260	0.2304	0.2148	0.0163

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[1] N. F. VII indicates that calamine cannot contain more than 2% ferric oxide. There is 80 Gm. of calamine in 1 L. of Calamine Lotion or (80 × 2% = 1.6 Gm. of ferric oxide). One liter of Calamine Lotion weighs 1092.4 Gm. Therefore, the maximum quantity of ferric oxide that may be present is (1.6 ÷ 1092.4 = 0.0014 Gm. = 1.4 mg.).

The Determination of Estrone in Oil Solutions of Estrogens*

By JONAS CAROL and FELICE A. ROTONDARO†

A method is described for the determination of estrone in oil solutions of natural estrogens. The procedure is based on the extraction of estrogens by immiscible solvents, separation of the ketone fraction with Girard Reagent T, followed by the spectrophotometric determination of estrone. The results of analysis of a series of control samples are reported.

PHARMACEUTICAL preparations containing natural estrogens dissolved in vegetable oils are widely used in the treatment of menopause and other conditions in which there may be an estrogen deficiency. Many of the preparations containing these hormones are made commercially from pregnant mares' urine and consist, on a weight basis, chiefly of estrone (1, 2) with minor amounts of estradiol, equilin, equilenin, and hippulin. From a regulatory or control standpoint it is highly desirable to have an accurate and reasonably rapid method for the determination of estrone in these preparations.

The literature contains many methods for the determination of estrogens. Kober (3, 4) estimated total estrogens colorimetrically using phenolsulfonic acid, and later naphtholsulfonic acid as a reagent. Szego and Samuels (5) modified the Kober procedure by substituting potassium guaiacolsulfonate for phenolsulfonic acid. Talbot *et al.* (6) determined phenolic ketosteroids colorimetrically by coupling with diazotized dianisidine after a preliminary separation with Girard Reagent T (7). Wolfe, Hershberg, and Fieser (8) developed polerographic methods for estrone and other ketosteroids. Hughes (9) determined a number of ketosteroids gravimetrically as a mercuric iodide complex with Girard Reagent T.

Veitch and Milone (10) determined estrone colorimetrically as the 2,4-dinitrophenylhydrazone. Hogness, Sidwell, and Zscheile (11) made a careful study of the absorption spectra of estrone and related compounds in order to obtain data for their quantitative estimation. Elvidge (12) proposed a spectrophotometric method for the determination of estrone in tablets by its absorption in alcoholic solution at 280 mμ. Chevallier and Manuel (13) determined total estrogens in urine by absorption measurements at 300 mμ using alcoholic solutions of *p*H 9.5.

After a study of the papers listed above, a method was devised based on (*a*) the extraction of phenolic steroids from the vegetable oil solutions by immiscible solvents, (*b*) separation of estrone and other phenolic ketosteroids by a semi-micro adaptation of Girard's reaction, (*c*) determination of the separated material as estrone by ultraviolet absorption spectrophotometry. Preliminary analysis by the proposed method indicated that more accurate results could be obtained using dilute aqueous sodium hydroxide solutions instead of alcoholic solutions for the spectrophotometric measurements.

EXPERIMENTAL

The absorption spectrum of estrone was determined, using a Beckmann Model D spectrophotometer, by making extinction measurements from 220–320 mμ of a solution containing 3 mg. U. S. P. reference standard estrone (dried in vacuum) per 100 ml. 0.1 *N* sodium hydroxide solution. The absorption spectrum shown in Fig. 1 has well-defined peaks at 239 mμ and 293 mμ.

To test the applicability of the Beer-Lambert law to solutions of estrone in 0.1 *N* sodium hydroxide, extinction measurements were made of a series of solutions containing 0.25–5.0 mg. estrone per 100 ml. at 239 mμ and 293 mμ. These data are recorded in Table I.

The straight lines produced in Fig. 2 by plotting extinction values against concentration demonstrates that the Beer-Lambert law is satisfied at both wave lengths. Quantitative measurements could be made at either 239 mμ or 293 mμ. The

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TABLE I.—APPLICABILITY OF THE BEER-LAMBERT LAW TO SOLUTIONS OF ESTRONE IN 0.1 *N* NaOH

Estrone, Mg./100 Ml.	$E_{1\ cm.}$ 239 mμ	$E_{1\ cm.}$ 293 mμ
0.25	0.089	0.028
0.50	0.166	0.056
1.00	0.330	0.110
2.00	0.667	0.218
3.00	...	0.334
5.00	...	0.550

quantity of estrone normally obtained by the following method makes measurements at 293 mμ most desirable to avoid unnecessary dilution of the final sample solution.

Apparatus.—A spectrophotometer suitable for measuring absorption in the ultraviolet. Two matched 1-cm. quartz absorption cells.

Reagents.—

Skellysolve C or high boiling petroleum ether.
Dilute sodium hydroxide solution—2 *N* (approximate).
Sodium carbonate solution—10% *w/v*.
Dilute sulfuric acid solution—50% *v/v*.
Ether—U. S. P. grade freshly washed twice with an equal volume distilled water.
Girard Reagent T (Trimethylacethydrazide Ammonium Chloride).
Glacial acetic acid—A. R. grade.
Chloroform—U. S. P. grade.
Methyl alcohol (absolute)—C. P. grade.
Estrone—U. S. P. Reference Standard.

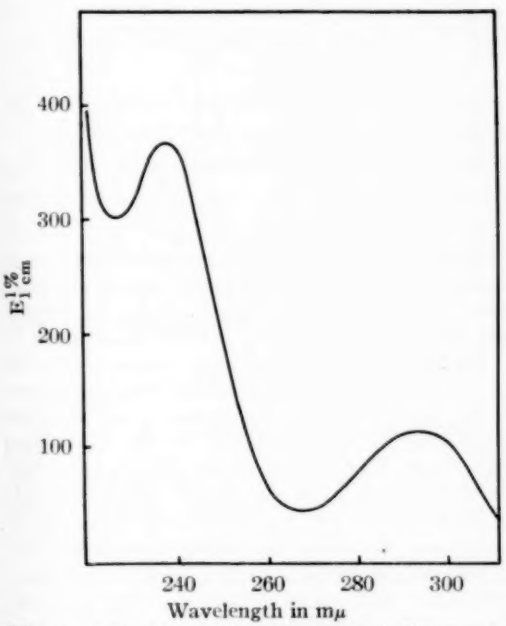

Fig. 1.—The Absorption Spectrum of Estrone in 0.1*N* NaOH

Standard Solution.—Dissolve ca. 25 mg. estrone (accurately weighed) in methyl alcohol, transfer to a 100-ml. volumetric flask, and dilute to volume with methyl alcohol. Pipette a 5-ml. aliquot of the alcoholic solution into a 50-ml. flask and evaporate to dryness on a steam bath using a current of air. Dissolve the residue by shaking vigorously with 2.5 ml. dilute sodium hydroxide and ca. 5 ml. distilled water. Dilute to volume with distilled water. The strong standard solution will keep indefinitely in a tightly stoppered bottle. The dilute standard should be freshly prepared at the time of analysis.

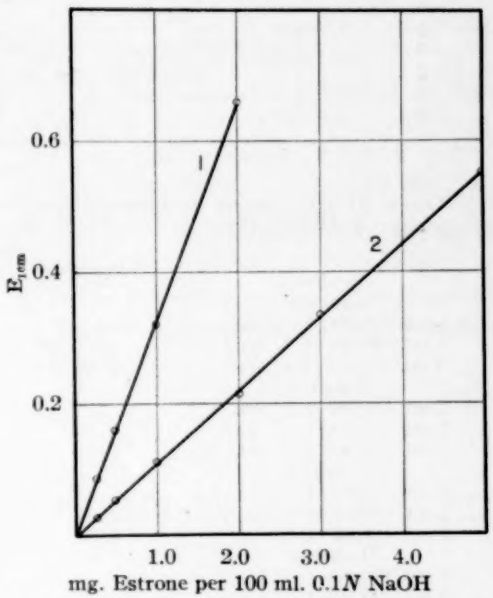

Fig. 2.—Plot of E Against Concentration at (1) 239 mμ and (2) 293 mμ.

Method.—Measure a portion of the sample containing ca. 1–10 mg. estrone and transfer to a 125-ml. separatory funnel containing 25 ml. Skellysolve C. Add 10 ml. dilute sodium hydroxide solution, shake vigorously for two minutes, and allow the two layers to separate completely. Transfer the aqueous layer to a second 125-ml. separatory funnel. Repeat the extraction with two additional 10-ml. portions of dilute sodium hydroxide solution adding each to the second funnel. Discard the Skellysolve solution. Add dilute sulfuric acid solution to the combined alkaline solutions until a permanent opalescence, or precipitate forms (acid to litmus). Cool thoroughly, add 25 ml. washed ether, shake carefully for one minute and allow the two layers to separate. Transfer the acid layer to a second 125-ml. separatory funnel and repeat the extraction with another 25 ml. of washed ether. Discard the acid layer. Extract the ether layers in succession with two 5-ml. portions of dilute sodium carbonate solutions and two 5-ml.

portions of distilled water. Discard the aqueous layers. Transfer the ether solutions to a 25-ml. beaker and evaporate to dryness on a steam bath in a current of air.

Dissolve the ether extract in a small amount of chloroform, warming if necessary, and transfer with the aid of a few milliliters of chloroform to a 20 x 150-mm. test tube. Carefully evaporate the chloroform on a steam bath in a current of air. Add 100 mg. Girard Reagent T and 0.5 ml. acetic acid to the test tube, cork loosely, and heat in a boiling water bath for two minutes. At the end of the heating period cool the test tube in an ice bath. After cooling, dissolve the reaction mixture in a few milliliters of ice water and transfer to a 125-ml. separatory funnel containing ca. 25 ml. ice water. Neutralize the solution to a *p*H 6.5–7.0 by the addition of dilute sodium hydroxide solution (about 3.0 ml.), and extract at once with three successive 15-ml. portions of chloroform. Combine the chloroform extracts in a second separatory funnel and wash with 5 ml. distilled water. Discard the chloroform and add the wash water to the aqueous solution in the first funnel. Acidify the solution with 2 ml. dilute sulfuric acid solution and allow to remain at room temperature for two hours. Add 15 ml. chloroform, shake vigorously for one minute and allow the layers to separate. Transfer the chloroform layer to a second separatory funnel. Repeat the extraction with three additional 15-ml. portions of chloroform. Wash the combined chloroform extracts with 5 ml. distilled water, filter through a pledget of cotton wet with chloroform, into a tared 25-ml. beaker, and evaporate to dryness on a steam bath in a current of air. Cool in a vacuum desiccator and weigh residue as estrone. If the sample contains 5 mg. or more of estrone this weight is satisfactory for the calculation of the estrone content of sample. If the estrone weighs less than 5 mg. it may be determined spectrophotometrically.

Dissolve the estrone residue in a small amount of methyl alcohol and transfer to a volumetric flask having a capacity that will produce a solution containing ca. 2–5 mg. estrone per 100 ml. Evaporate to dryness on a steam bath in a current of air. Add ca. 5 ml. dilute sodium hydroxide solution, 10 ml. distilled water, shake vigorously until the residue is completely dissolved, and dilute to volume with distilled water. Prepare a blank solution containing the same concentration of sodium hydroxide. Determine the extinction (E) of the sample solution, relative to the blank at 293 mμ. Determine the

TABLE II.—RESULTS OF ANALYSIS OF CONTROL SAMPLES OF ESTRONE IN SESAME OIL

Sample	Sesame Oil, Ml.	Estrone Added, Mg.	Estrone: By Residue, After Girard Separation, Mg.	Estrone: By Residue, After Girard Separation, % Recovery	Estrone: By Spectrophotometer, Mg.	Estrone: By Spectrophotometer, % Recovery
1	5.0	..	0.0	0	0.0	0
2	5.0	1.00	1.1	110	1.09	109
3	5.0	2.87	2.8	98	2.8	98
4	5.0	5.57	5.7	102	5.6	101
5	5.0	8.90	8.7	97	8.8	98

TABLE III.—RECOVERY OF ESTRONE ADDED TO COMMERCIAL SAMPLES OF ESTROGENS IN OIL

Sample	Estrone Added, Mg.	Estrone: By Residue After Girard Separation, Mg.	Estrone: By Residue After Girard Separation, % Recovery	Estrone: By Spectrophotometer, Mg.	Estrone: By Spectrophotometer, % Recovery
(Labeled)					
5 ml. (10,000 I. U./cc.)	..	2.0	...	1.8	...
5 ml. (10,000 I. U./cc.)	6.77	8.0	91	8.0	93
5 ml. (10,000 I. U./cc.)	7.36	8.5	91	8.7	94
(Labeled)					
5 ml. (10,000 I. U./cc.)	..	1.1	...	1.1	...
5 ml. (10,000 I. U./cc.)	2.35	3.3	96	3.2	93
5 ml. (10,000 I. U./cc.)	7.89	8.8	98	8.7	97
(Labeled)					
5 ml. (10,000 I. U./cc.)	..	4.3	...	4.2	...
5 ml. (10,000 I. U./cc.)	1.04	5.4	101	5.3	100
5 ml. (10,000 I. U./cc.)	4.96	9.2	100	9.1	99
(Labeled)					
5 ml. (20,000 I. U./cc.)	..	6.4	...	6.4	...
5 ml. (20,000 I. U./cc.)	..	6.4	...	6.5	...
5 ml. (20,000 I. U./cc.)	1.93	7.9	95	76.	91
5 ml. (20,000 I. U./cc.)	2.67	8.7	96	8.4	92
(Labeled)					
5 ml. (20,000 I. U./cc.)	..	9.4	...	9.4	...
5 ml. (20,000 I. U./cc.)	..	9.4	...	9.4	...
5 ml. (20,000 I. U./cc.)	3.22	12.5	99	12.4	98
5 ml. (20,000 I. U./cc.)	4.04	13.4	100	13.4	100

extinction (E) of the standard solution, relative to the blank, at 293 mμ.

$$\text{Mg. estrone in residue} = V\left(\frac{E \text{ sample} \times W}{100\ E \text{ standard}}\right)$$

where

W = mg. estrone per 100 ml. standard solution.
V = volume in ml. of sample solution.

A series of samples were prepared by dissolving weighed quantities of estrone in methyl alcohol, adding 5 ml. of Sesame Oil N. F. to each, and heating on a steam bath until the alcohol was evaporated. The results of analysis of these samples are shown in Table II.

By the same procedure, weighed amounts of estrone were added to a series of commercial samples of natural estrogens in oil. These samples were analyzed before and after the addition of estrone and the results are given in Table III.

SUMMARY AND CONCLUSIONS

1. A gravimetric method for the determination of phenolic ketosteroids, as estrone, in commercial oil solutions of natural estrogens is described for samples containing 5 mg. or more of these substances. Samples containing less than 5 mg. of ketosteroids may be analyzed by the application of the spectrophotometric method to the residues obtained by the above method.

2. Results of analysis of a number of control samples show good agreement between checks and good recovery of estrone added to commercial samples of natural estrogens in oil.

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A Chemical Study of Two Isomeric Sodium Hydroxymercuribenzoates*

By THOMAS J. HALEY,† L. D. EDWARDS,‡ and C. H. JOHNSON,§,‖

A RECENT work on dental caries by Hanke (1) showed that sodium *p*-hydroxymercuribenzoate in the concentration of 1:5000 was a very effective oral germicide. The wide use of this solution in some localities has led to this investigation of both the *ortho* and the *para* isomers of sodium hydroxymercuribenzoate.

EXPERIMENTAL

Synthesis of *o*-Anhydrohydroxymercuribenzoic Acid.—This compound, prepared by the method of Whitmore and Culhane (2), was obtained in 85% yield. It melted at 325° with decomposition. It was converted into the sodium salt by dissolving it in hot diluted sodium hydroxide solution and then chilling the solution to precipitate the salt.

Derivatives of *o*-Anhydrohydroxymercuribenzoic Acid.—(*A*). The acid anhydride was converted into benzoic acid by boiling with concentrated hydrochloric acid according to the procedure of Dimroth (3). A mixed melting point with standard benzoic acid gave a melting point of 121°. (*B*). *o*-Chloromercuribenzoic acid was prepared by dissolving the acid anhydride in diluted acetic acid and then adding sodium chloride [Pesci (4)]. The product, recrystallized from ether, melted at 231° and gave a positive test for halogen. (*C*). *o*-Benzoylbenzoic acid was prepared by mixing the acid anhydride with excess bromine water containing a small amount of potassium bromide. This mixture was heated until colorless and the appearance of a precipitate. The

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product melted sharply at 127–128° after recrystallization from ether. Further characterization was carried out by converting this derivative into an anilide. This last compound melted at 195°, the melting point of the anilide of *o*-benzoylbenzoic acid. (*D*). An attempt was made to prepare a piperazonium derivative by the method of Pollard, Adelson, and Bain (5). This resulted in the breakdown of the C—Hg linkage giving metallic mercury.

Synthesis of Sodium *p*-Hydroxymercuribenzoate.—The following intermediates were required before the final product was obtained. Sodium *p*-toluenesulfinate was prepared by the method of Whitmore and Hamilton (6). This material was then converted into *p*-tolylmercuric chloride by the method of Whitmore, Hamilton, and Thruman (7). From this latter compound, *p*-chloromercuribenzoic acid was prepared by the method of Whitmore and Woodward (8). A yield of 85% was obtained. The purified product (melting point 272°) was dissolved in boiling diluted sodium hydroxide solution to form sodium *p*-hydroxymercuribenzoate. Considerable difficulty was encountered in precipitating this salt because absorption of carbon dioxide from the air caused a breaking of the C—Hg linkage. Further, when recrystallization was carried out using 50% ethanol the breakdown was even more rapid. Breakdown also occurred in a capped vessel at 10° in the icebox. Similar results were obtained when acetone or carbon tetrachloride was substituted for ethanol. Under the same conditions the *ortho* isomer reacted similarly. These difficulties reduced the final yield of the *para* compound to about 10%.

Derivatives of Sodium *p*-Hydroxymercuribenzoate.—(*A*). The salt, when heated to boiling with concentrated hydrochloric acid, yielded benzoic acid (2). A mixed melting point with standard benzoic acid gave a melting point of 121°. (*B*). A solution of the salt was mixed with bromine water and a small amount of potassium bromide and boiled until colorless (2). This solution was extracted with ether and the resulting product gave a positive Beilstein's test. This derivative was *p*-bromobenzoic acid melting at 251°. (*C*). A 5% solution of iodine in 95% ethanol was added to a solution of the salt until a yellow color remained. The mixture was heated until colorless, cooled, filtered, and then extracted with ether. Platelets from the ether solution were recrystallized from water. The *p*-iodobenzoic acid formed melted at 269° [Whitmore and Woodward (9)].

Analysis of the isomers for mercury.—The *para* compound was analyzed using the U. S. P. XII Method (10) adapted for micro quantities. Aqueous solutions of this isomer were digested with nitric acid at 75–80° until clear. The clear solutions were titrated with a solution of ammonium thiocyanate (1 cc. = 0.0811 mg. of Hg), using ferric alum indicator. The results obtained are shown in Table I. As this method gave variable results and the exact end point was hard to determine, the *ortho* isomer was not analyzed with thiocyanate.

A quantitative spectrographic analysis of both isomers was then undertaken using a B & L Litrotype Spectrograph. The instrument was set in the G3 position and a standard plate exposed. Traces of the following elements were found:

Ortho isomer—Cu, Fe, Al, Mg, Mn, Si, Ca, and Na.
Para isomer—Cu, Fe, Al, Mg, Mn, Si, Ca, and Na.

A standard mixture was made up from mercuric acetate, sodium chloride, and carbon. Samples of 0.5 Gm. each were taken as follows:

Standard #1—4% mercury, intensity equivalent to 80% mercury.
Standard #2—2.5% mercury, intensity equivalent to 50% mercury.
Standard #3—1.5% mercury, intensity equivalent to 30% mercury.

A 0.5-Gm. sample was made up by mixing 25 mg. of the unknown with sodium chloride and carbon. This gave a final dilution of the unknown of 5% of the mixture.

TABLE I.—COMPARISON OF THE RESULTS OF THIOCYANATE AND DITHIZONE TITRATIONS

Sample		Calcd. Amount of Mercury, Mg.	Exptl. Found Mercury, Mg.	% Mercury
Thiocyanate				
Para	1	0.222	0.138	62.30
	2	0.222	0.130	58.70
	3	0.222	0.105	47.50
	4	0.222	0.105	47.50
	5	0.154	0.081	52.84
	6	0.154	0.081	52.84
	7	0.154	0.081	52.84
Dithizone				
Ortho	1	0.067	0.0609	90.47
	2	0.067	0.0536	79.67
	3	0.067	0.0609	90.47
	4	0.067	0.0582	86.42
	5	0.067	0.0609	90.47
	6	0.067	0.0609	90.47
Para	1	0.0818	0.0573	70.03
	2	0.0818	0.0564	68.92
	3	0.0818	0.0609	74.48
	4	0.0818	0.0791	96.71
	5	0.0818	0.0782	95.60
	6	0.0818	0.0791	96.71
Blank		0.0000	0.0000	00.00

The spectrograph was now set in the G3 position and the Lanthanum standard was added to the arc electrodes, after which the samples were run. The lines, Mercury (4358.4 Å.) and Lanthanum (4333.7 Å.), were read on the microdensitometer. This gave the ratio of intensity of the Hg:La lines. An appropriate background correction was made, and conversion to light intensity readings was made by calibrating the spectrographic plate with a rotating log stop sector. The graph of ratio of intensity against per cent mercury was plotted on log-log paper. The per cent of mercury in the sample was obtained directly from this graph (Fig. 1). Table II shows the results obtained.

TABLE II

Sample	Ratio of Intensity	% Mercury
1	16.50	80
2	11.75	50
3	8.80	30
Para isomer	9.30	33
Ortho isomer	10.03	38

In order to obtain more accurate results, an adaptation of the Eckert (11) method using dithizone titration of mercury was employed. The only serious drawback of the method was the combining of the dithizone reagent with micro quantities of copper, obscuring the end point. This was overcome by redistilling all the water used in a glass-distillation apparatus.

A dithizone solution was made up to contain 12.5–13.0 mg. of the reagent per 500 cc. of carbon tetrachloride. This was standardized with a known mercury standard (1 cc. equivalent to 0.01 mg. of mercury).

A sample of the mercurial was placed in a 50-cc. round-bottomed flask, a West condenser attached, and the material digested with 5 cc. of concentrated nitric acid on a water bath (75–80°) for fifteen hours. At the end of this time the sample was transferred to a 150-cc. separatory funnel. The flask and the condenser were washed with 25 cc. of water. The washings and 5 cc. of a 20% hydroxylamine sulfate solution were added to the digested sample in the funnel and the volume made up to 125 cc. with distilled water. This solution was then titrated with dithizone standard in the manner used by Eckert.

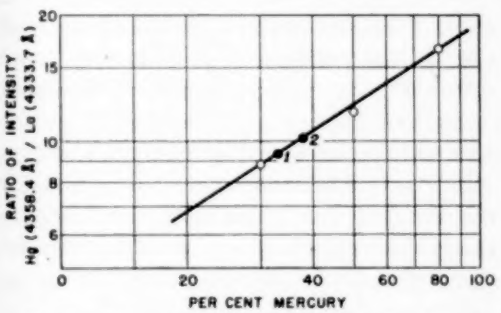

Fig. 1—O-samples 1, 2 and 3; ●-*1* the *para* isomer; ●-*2* the *ortho* isomer.

Both the *ortho* and *para* isomers were analyzed six times, a blank being analyzed at the same time to check the reagents used. The results obtained by this method show a good correlation with the calculated amount of mercury in each of the isomers. Table I shows the results obtained with both the thiocyanate and the dithizone methods and a comparison of the results obtained shows the latter to be more accurate in the determination of micro quantities of mercury.

SUMMARY

1. Two isomeric sodium hydroxymercuribenzoates and several of their derivatives were prepared by standard procedures.

2. *o*-Benzoylbenzoic acid, a new derivative for the characterization of sodium *o*-hydroxymercuribenzoate, was prepared.

3. Piperazine hexahydrate caused a breakdown of the C—Hg linkage and cannot be used to characterize organic mercurial acids.

4. Both isomers were extremely unstable in the presence of alkali and carbon dioxide. Also under the same conditions organic solvents such as ethanol, acetone, and carbon tetrachloride caused a break in the C—Hg linkage giving rise to metallic mercury.

5. Both isomers were analyzed spectrographically, and although this method was not the most accurate one employed, it could be used for determining micro quantities of mercury when an estimation of the mercury content of an organic compound was desired. Further, it is possible to detect micro quantities of impurities which would not be detected in other methods of analysis.

6. The most sensitive and accurate method of determining mercury in micro quantities is by dithizone titration. This method is more accurate than the thiocyanate titration because no indicator is necessary and the end point is obtained only when there is no mercury left to react with the reagent. Another advantage of the dithizone method is the extremely small quantity of sample required for an analysis; this is of prime importance when dealing with organic mercurials whose solubility is limited.

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Studies in the Preservation of Liquid Pharmaceutical Preparations

I. The Relationship Between the Preservative Action of Alcohol and the Solid Content of the Preparation*

By I. W. GROTE and PEGGY WALKER

PRIOR to the discovery of the art of distillation by the Arabs in the 9th century, liquid medicinal preparations were often put up as thick, viscous solutions containing honey and were called electuaries or treacles. A few preparations which depended on a high salt content for preservation were dispensed. With the discovery of the art of distillation and the general availability of alcohol of a strength sufficient to keep in air, this solvent and preservative became of great importance in the art of the pharmacist. It had several advantages over sugar and salt as a preservative. It more effectively extracted the active medicinal principles from the roots, herbs, etc., used; it would extract and hold in solution the various oils used as ingredients and, if taken in sufficient amounts, it greatly added to the efficacy of the drugs in the preparation by simultaneously granting a sense of well-being to the patient. Many of the earlier formulas contained both sugar, largely as honey, and alcohol. These were called cordials, elixirs, or crèmes. In some cases wine was used as the extractive agent, but even in these cases alcohol and sugar were often added as further preservatives, since the ancient wines were often of low alcoholic content. In more recent time, starting in the 17th century, it became more general to extract the drug material with strong or diluted alcohol or brandy and prepare the product as a tincture. Often this tincture was concentrated further in a still to give the fluid or solid extract.

Gradually, through the centuries, and solely through trial-and-error procedure, the pharmacist learned that certain combinations of drug, sugar, and alcohol would keep without fermentation or mold growth. These early preparations and wines were often further protected in the storage jar or bottle by overlaying with olive oil, which effectively shut out the air and prevented vinegar formation and mold growth. This method of adding oil is still used to preserve low-alcohol Italian wines as well as urine samples in the physiological chemistry laboratory. With the invention of the cylindrical bottle in the late 17th century, and the introduction of the cork stopper into more general use a little later, it became possible to prepare medicinal preparations on a large scale for general sale in the pharmacy (1). There was a corresponding necessity that the preparations keep free from fermentation and mold growth over a longer period.

A survey of the literature in both pharmaceutical texts and scientific journals shows but few scientific studies pertaining to the amount of alcohol needed as a preservative for medicinal preparations in spite of the tremendous amount of empirical work that has been undoubtedly carried out on the subject. Unfortunately, most manufacturing concerns consider this type of information a "trade secret." Since such studies involve shelf testing or storage over a period of months and years they are not suitable for a bachelor's or master's thesis in a college and are considered beneath the dignity of a doctor's thesis. They are too "practical."

In spite of the meager literature on the subject, however, we have found it is generally accepted in the drug trade that to be absolutely safe some 15 per cent of alcohol is required for a slightly acid preparation (*p*H 5.5 or below) while a neutral or slightly alkaline solution (above *p*H 5.5 to *p*H 8.5) requires 17 to 18 per cent.

Below *p*H 3.0 and above *p*H 11.0 the hydrogen and hydroxyl ions show a marked preservative action toward most yeasts.

* Received Jan. 19, 1946, from the Research Laboratories of the Chattanooga Medicine Company, Chattanooga, Tenn.

mold, and bacteria (including most of the pathogenic types). High salt concentrations show a similar preservative action. Microbiological growths can occur, however, at extremely low and extremely high *p*H as well as in high salt concentrations. Examples are the growths occurring in the sulfuric acid pickling tanks used for the cleaning of steel, the growth occuring in various reagent solutions on the chemist's shelves, including copper sulfate, sodium arsenite, alkaline sodium phosphate, and many others. Many microbiological growths do not produce marked odors, gases, or other evidences of their presence beyond the formation of insoluble debris which floats about in the solution. Most often these are not recognized as organized growths but are dismissed as "chemical precipitates." Often they are hidden and mixed with real chemical precipitates. Drug deterioration on standing is due to microbiological growths much more often than is generally realized.

Gabel's study of "The Relative Action of Preservatives in Pharmaceutical Preparations" (2) bears out the generally accepted idea that 15 to 17 per cent alcohol is required for safe preservation of medicinal preparations. Gabel used three types of media: an acid solution containing a 6 per cent senna extract and 6 per cent sugar (which would have a *p*H of about 5.0–5.5); an alkaline solution containing 6 per cent beef extract, 6 per cent sugar and sodium bicarbonate (about *p*H 8.0); and a neutral one containing only 6 per cent beef extract and 6 per cent sugar (about *p*H 6.0 to 6.5). Each solution was tested separately with varying amounts of preservative, placed in fermentation tubes and inoculated with a gas-producing bacterium.

The tests were allowed to run from one to two months at a slightly elevated temperature. The results found in the case of glycerin and alcohol were as follows: a 45 per cent solution of glycerin molded in acid and neutral media but kept in the alkaline solution. No data for a satisfactory preservation with glycerin alone, either in acid or neutral solution, were given. We have found in the trade, however, that approximately 50 per cent glycerin by volume is regarded as safe. In acid solution 15 per cent alcohol by volume was sufficient to prevent molding or fermentation, while 17.5 per cent alcohol was required to keep both the basic and neutral solutions.

Perry and Beal (3) used positive inoculation of sterile 1 per cent dextrose broth with pure cultures of specific organisms, namely; *Saccharomyces cerevisiae* and *Penicillium glaucum*, to find the exact percentage of alcohol necessary to inhibit the growth of the organisms and also the amount required to destroy them completely. After inoculation, the fermentation tubes were allowed to stand at room temperature for from four to eight days. The results from alcohol were as follows: when the tubes were covered so as to prevent evaporation, those inoculated with *Saccharomyces cerevisiae* showed gas formation up to a concentration of 11 per cent alcohol by volume. No living organisms were found at 15 per cent. The tubes inoculated with *Penicillium glaucum* showed growth up to 8 per cent and no living organisms were found above 14 per cent. Where the tubes were not capped, the results were not uniform and a higher concentration of alcohol was required, 18 per cent to prevent gas formation; 20 per cent to kill *Saccharomyces cerevisiae;* 8 per cent to prevent visible growth of mold; and 17 per cent to destroy completely *Penicillium glaucum*.

At the request of the British Pharmacopœia Commission, Bullock and Elsdon made a very careful study of the preservation of fresh and concentrated infusions (4, 5). This study was made with a view of saving alcohol as compared to the amounts needed to prepare either tinctures or fluidextracts. In place of the usual method of testing the keeping qualities of a medicinal preparation by storing it for weeks or months, these authors ran bacteria and mold counts by the methods used for sewage and milk and reported at the end of various periods if the count was increasing or decreasing. They found that the amount of preservative required in each case is a separate problem according to the nature of the drug infused. They also found that yeasts are more easily

killed by alcohol than bacteria or molds. Acid conditions kill bacteria but favor mold growth, which persisted even in concentrations of 1 per cent of strong hydrochloric acid. None of their preparations contained more than 20 per cent total solids and to none was sugar added. They found that due to the preservative help of the essential oils, clove and senna-ginger infusions would keep in 10 per cent alcohol concentration, but that 15 per cent was necessary to preserve other concentrated infusions, including the difficult ones of quassia and calumba, not containing marked amounts of essential oils.

No direct statement was found in the literature as to the effect of a relatively high extractive or sugar content on the alcohol requirement for safe preservation of a medicinal preparation. However, a study of the United States Pharmacopœia, National Formulary, and other pharmaceutical compendia in common use showed that the presence of a high solid content actually greatly reduces the need for alcohol in certain preparations. A study of proprietary preparations on the druggist's shelf, such as cough syrups, laxatives, expectorants, tonics, etc., shows that many are below 15 per cent alcohol in strength, but in such cases they are relatively high in sugar or plant extractives.

It occurred to us to make a study of the formulas of such medicinal preparations to see if any correlation existed between total solid content and the amount of alcohol needed to preserve. It seemed logical to assume that if a large part of the weight of a given solution is made up of solid or non-aqueous material, a smaller amount of alcohol would be required to preserve the solution. It was, of course, early recognized that preservation was not simply a matter of colligative properties, but it did appear that solid material dissolved in a solution had a marked effect in increasing the preservative action of alcohol. It was recognized further that it is not a simple matter of mathematics. If 20 per cent alcohol will preserve a neutral solution, leaving approximately 80 per cent water, replacement of 4 per cent of water by 4 per cent solids will not cut down the per cent of alcohol needed by 1 per cent, as required by the ratio, but certainly it will reduce the amount by part of a per cent.

In our speculations which led to this paper, and they are pure speculations, although some actual trade experiences have brought considerable confirmation to the idea, we have first assumed that sugar and glycerin have a sort of additive effect when used as preservatives in conjunction with alcohol. In other words, if sugar in the concentration of 850 Gm. per 1000 cc., or 65 per cent by weight, will just preserve itself as simple syrup, in figuring the alcohol content needed to preserve a given preparation containing sugar, a syrup equivalent of the sugar present could be first subtracted from the total volume and only 15 to 18 per cent alcohol by volume be used to preserve the remaining that we have called "free water."

If glycerin be present, the per cent of glycerin can be doubled and this amount subtracted to get the "free water" content, since 50 per cent glycerin solutions seem stable. In the case of preparations containing relatively high ionizable salt concentrations, not only must the actual amount of salt present be taken into consideration, but a further lowering of the "free water" content be ascribed to ion hydration.

This idea is not new, but we can find nothing in the literature to suggest our second assumption, that in preparations containing a considerable percentage of plant extractives or other solids, a further amount corresponding to the volume and nature of the material present, should be subtracted from the total volume to finally give what we call the "free water" content. The total of these subtractions often leaves a surprisingly small amount of "free water," so that an alcohol content of, in some cases, only 2–5% may be of great value in keeping the preparation.

As an example, if a preparation contained 425 Gm. of sugar and 300 Gm. of vegetable extractive per liter, the syrup equivalent of the sugar would equal 500 cc. and should be subtracted. Then the 300 Gm. of extractive would have a volume of about 250–300 cc., leaving a total of 200 to 250 cc. of "free water" present, which would necessitate

only 30 to 50 cc. of alcohol to preserve, or 3–5 per cent alcohol by volume of the preparation. Thus the 3 to 5 per cent of alcohol by volume so often present in cough syrups and expectorants can serve a very useful purpose although the alcohol is usually present by accident as it were, through employment of fluidextracts, tinctures, and flavoring agents in the manufacture of the preparations. This amount of alcohol is often regarded as a useless waste by the pharmaceutical chemist. We have found by experiment, however, that even 3 per cent of alcohol often prevents slow fermentation and mold growth that will take place in the identical preparation if this small amount of alcohol be omitted. Substitution of sugar by glucose could possibly lead to trouble, as we have found by experiment, unless a small amount of alcohol be added, since glucose does not in our hands seem to behave like sucrose, and should be subtracted as an "extractive" rather than counted as a sugar syrup equivalent.

This theory was then applied to a study of the various low alcohol elixirs, emulsions and syrups of the N. F., U. S. P., B. P., etc. Only those preparations which do not contain benzoic acid, salicylic acid, chloroform, or other well-recognized preservatives were considered. Those preparations having a total alcohol content of less than the 15 to 18 per cent regarded as the lower limit of safety, are shown in the accompanying tables.

The additive effect of sugar syrup as a preservative in conjunction with alcohol is recognized, in principle at least, by the fact that the U. S. P. suggests the addition of only 6 to 7 per cent of alcohol to various oil emulsions for conversion from extemporaneous preparations to stable ones. As these preparations contain 50 per cent oil and 10 to 20 per cent sugar syrup, the alcohol content based on the "free water" present in the emulsion will raise this amount to over 20 per cent. In the U. S. P. preparations given in the twelfth edition, all the apparently low alcoholic solutions, when recalculated from the standpoint of subtracting the solids, 50 per cent glycerin solution, and sugar syrup present, show that the alcohol content based on the water present is above the 15 to 18 per cent required for safety.

The N. F. preparations of the seventh edition show six below the lower limit of 15 per cent even when recalculated from a "free water" content. Of these, the elixir of benzaldehyde can be excused from consideration, since benzaldehyde itself is a recognized antiseptic.

The elixir of ammonium bromide, containing about 5.8 per cent alcohol, contains only 9 to 11 per cent alcohol based on water content alone. However, it also contains 0.1 per cent of essential oil such as orange, lemon, coriander, and anise, which have a known preservative action, as well as 8.5 per cent of inorganic ammonium bromide, which has an ionizable-salt preserving action. The cumulative action of all three types of preservatives must be regarded as in force here as well as in the case of the elixir of sodium bromide and of potassium bromide which have a still higher salt content.

The compound elixir of vanillin is probably partially preserved by the relatively large amounts of essential oils of cinnamon, cardamon, and orange present. In the case of mineral oil emulsions, a different picture is presented, due to the high percentage of the oil present. As we mentioned in our historical introduction, the preservative action of covering with oil has been known from antiquity and in the case of an oil emulsion, each droplet or interface of aqueous liquid can be considered as being somewhat protected by the oil.

In reviewing the Pharmaceutical Recipe Book, second edition, it was noted that six preparations of interest to us contained less alcohol than is considered safe. Of these, Mattioli's elixir, containing only 12.8 per cent alcohol based on water; emulsion of santal oil, of 8.8 per cent alcohol based on water; and mixture of Guaiac, containing 12.9 per cent alcohol on the same basis, were found to contain cinnamon oil, a very powerful germicide, and were thereby excused from further explanation.

The Irish moss emulsion of cod-liver oil with hypophosphites, containing 7 per cent

TABLE I—OFFICIAL AND NONOFFICIAL COMPOUNDS IN COMMON USE

Preparation	Syrup, 50% Glycerin and Extractives, %	"Free" Water Content, %	Total Alcohol, %	Alcohol Content Based on Free Water Content, %
United States Pharmacopœia				
Syrup of Citric Acid	98	1.3	0.7	Over 20
Syrup of Orange	97	None	3.7	Over 20
Syrup of Tolu Balsam	96.5	None	2- 4	Over 20
Syrup of Glycyrrhiza	75	20	5	20
Syrup of Ipecac	93	5	2	Over 20
Syrup of Wild Cherry	94.6	3.5	2	Over 20
Aromatic Syrup of Rhubarb	85	8.5	6.5	Over 20
Cmpd. Syrup of Sarsaparilla	76.5	13.2	10.2	Over 20
Syrup of Senna	75	18.7	6.2	Over 20
Cod Liver Oil Emulsion	72.5	23	4.5	16.5
Mineral Oil Emulsion	72.5	21.5	6.0	Over 20
Elixir Phenobarbital	60.0	25–28	12–15	Over 20
National Formulary				
Elixir Ammonium Bromide	41.1	53–55	5.86	About 9
Elixir Potassium Bromide	43.7	52	5- 7	About 9
Elixir Sodium Bromide	43.7	52	5- 7	About 9
Cmpd. Elixir Benzaldehyde	40	55	5	About 9
Elixir of Glycerophosphates	74.6	18.4	7	Over 20
Cmpd. Elixir Cardamom	40.2	50	9.5	16
Elixir Cascara Sagrada	37.5	53.3	11.2	17.5
Elixir Gentian	33	53	14–17	Over 20
Low Alcoholic Elixir	37.5	34	8–10	19
Elixir of Pepsin	42.2	45	13–15	Over 20
Elixir of Pepsin and Bismuth	44.6	42.4	9–11	17.7
Cmpd. Elixir of Vanillin	33	57.3	9	13.5
Cod Liver Oil Emulsion	64.5	28	7.5	Over 20
Emulsion C. L. O. with Egg	60	30	8- 9	Over 20
Emulsion of Petrolatum	55.5	38.5	6	13.5
Syrup of Althea	92	5	2- 3	Over 20
Syrup of Asarum	82	1- 4	14–17	Over 20
Syrup of Cacao	94	None	2- 3	Over 20
Syrup of Cherry	94	4- 5	1- 2	Over 20
Syrup of Cinnamon	94	4- 5	1- 2	Over 20
Syrup of Ephedrine	92	4- 5	1- 2	Over 20
Arom. Syrup of Eriodictyon	94	4- 5	6.8	Over 20
Syrup of Ipecac and Opium	91	4- 5	1- 2	Over 20
Syrup of Raspberry	94	4- 5	1- 2	Over 20
Syrup of Rhubarb	83.6	9	5- 7	Over 20
Compd. Syrup of Squill	84.5	8- 9	7- 8	Over 20
Syrup of Senega	79	10–11	9–10	Over 20
Pharmaceutical Recipe Book				
Elixir of Anise	62.5	31	5.85	16
Mattioli's Elixir	10.5	77.8	11.3	12.8
Emulsion of Castor Oil	55	32	8	20
Emulsion of Cod Liver Oil	78.8	14.2	7	Over 20
Irish Moss Emulsion of Oil	51.3	42	7	14.3
Emulsion of Petrolatum	67.5	24.5	8	Over 20
Emulsion of Liquid Petrolatum	55	39	6	13.3
Emulsion of Santal Oil	20	75	7	8.8
Mixture of Guaiac	25	65.3	9.7	12.9
Cmpd. Syrup of Cimcifuga	76	19	4.5	19.5
Cmpd. Syrup of Codeine	92.5	4	2.35	Over 20
Cmpd. Syrup of Senna	72.6	15.4	12	Over 20
British Pharmacopœia				
Elixir Cascara Sagrada	60	29	11	Over 20
Syrup of Orange	91	None	9	Over 20
Syrup of Lemon	94	None	6	Over 20
Syrup of Ginger	96	None	4	Over 20
British Pharmaceutical Codex				
Elixir of Aletris	18	68.1	13.9	16
Elixir of Ipecac	52	38.2	9.8	Over 20
Elixir of Ovo-Lecithin	75.3	59.6	1.1	2
Syrup of Poppy	87.5	10	3.1	Over 20
Syrup of Rhubarb	93	2.8	4.2	Over 20
Syrup of Red Poppy	85	10.5	4.5	Over 20
Syrup of Lemon Juice	89.5	6.9	3.6	Over 20

alcohol total, equivalent to about 14 per cent based on the water content, is close enough to the limit of safety that the 20 per cent salt content of sodium, calcium, and potassium hypophosphite plus the oil preservative action would provide the margin of safety.

The British Pharmacopœia of 1932 described only four preparations of interest to our study, and all four met the alcohol requirement very readily.

The British Pharmaceutical Codex of 1934 contains a number of preparations of low alcohol content, but an extensive use of chloroform as an additional preservative was noted, in marked contrast to the few chloroform preparations found in American practice. Of the very few low-alcohol, non-chloroformic preparations found, one requires comment; the others contained the theoretically correct amount. A preparation called elixir of ovolecithin, according to our calculations, should spoil, since it contains sufficient glycerin to preserve only 70 per cent of the solution, the only additional preservative being 2 per cent of alcohol. However, we are not familiar with the actual experience with this purely British product.

In the several months since the idea embodied in this paper occurred to us, we have made a number of preliminary experiments using various amounts of plant extractives, sugar syrup, and water with the calculated amounts of alcohol which would just preserve and that which would just allow mold growth and fermentation. These solutions were put in fermentation tubes, inverted test tubes, or bottles, and inoculated with actively spoiling vegetable extractives. It is too early to report a final outcome but the results so far have not been contradictory. The results obtained from these experiments will be reported in a further communication. We are presenting this preliminary paper at this time, however, because the idea does seem to offer a starting point for calculation of amounts of alcohol to be used for an experimental study of the keeping qualities of a proposed liquid medicinal preparation of high solid content.

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Book Review

Surface Active Agents, by C. B. F. YOUNG and K. N. COONS. Chemical Publishing Company, Brooklyn, 1945. x + 381 pp. 13.5 x 21.5 cm. Price, $6.00.

Surface Active Agents is an attempt to bring together the pertinent facts regarding these agents, or compounds.

Part I is general in character and introduced by a chapter on the theory of surface tension which adds nothing not existing already in many elementary texts. The chapter on determination of surface tension, in this same category, contains a few incorrect statements and neglects to discuss interfacial tension at all. However, Part I of this volume contains the most complete and most valuable cataloguing of surface active agents ever assembled in one place. The list is alphabetized by trade names followed by the chemical composition, a brief statement of industrial use, and the manufacturer's name. To many readers this list alone is worth the price of the book.

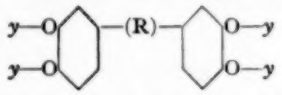
Part II, in which each chapter describes an industrial use for these compounds, is more specific in character. These chapters are too superficial to be of too much help. But the general purpose, the arrangement, the illustrations, and the typography are excellent.—MELVIN W. GREEN.

The Synthesis of Some Alpha, Omega-Bis-(3,4-Dihydroxyphenyl) Alkanes*,†

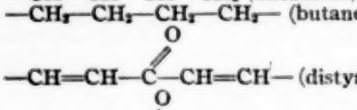
By OLE GISVOLD‡, DONALD BUELOW§, and EDWARD H. CARLSON

This paper reports the preparation of three α, ω-bis-(3,4-dihydroxyphenyl) alkanes. In preliminary tests these compounds have antiseptic and antioxidant activities which compare very favorably with those of nordihydroguaiaretic acid.

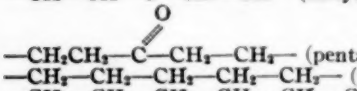
THE MARKED antiseptic (1) and antioxidant properties (2) of nordihydroguaiaretic acid, isolated from *Larrea divaricata* (3), suggested the synthesis of *α,ω-bis-*(hydroxyphenyl substituted) alkanes as possible useful antiseptics and antioxidants depending on the number and position of the hydroxy groups in the phenyl radicals, as represented by the following structure:



R = —CH=CH—CH=CH₂ (butadiene),
—CH₂—CH₂—CH₂—CH₂— (butane),



(distyryl ketone),



(pentane-3 one),
—CH₂—CH₂—CH₂—CH₂—CH₂— (pentane),
—CH₂—CH₂—CH₂—CH₂—CH₂—CH₂— (hexane),

y = H, CH₃—, CH₂,

It is well known that alkyl groups enhance the antiseptic activity of most phenols and much has been published in this field. However, little attention has been given to a study of the *α,ω-bis-*(hydroxyphenyl) alkanes. Richardson (4) prepared several *α,ω-bis-*(*p*-hydroxyphenyl) alkanes and reported that they were antiseptic, but only slightly soluble in water. Nordihydroguaiaretic acid is sufficiently soluble in water to kill *Staphylococcus aureus* upon contact. It is freely soluble in alcohol and sufficiently soluble in 25 per cent alcohol to prepare solutions many times stronger than necessary to kill *S. aureus*. It seemed reasonable to assume that the *α,ω-bis-*(*ortho* and *meta* dihydroxyphenyl) alkanes would be sufficiently water soluble to make them useful. This paper reports the preparation of three *α,ω-bis-*(*ortho* dihydroxyphenyl) alkanes. In preliminary tests these compounds have antiseptic and antioxidant activities that compare very favorably with those of nordihydroguaiaretic acid. The synthesis of the *α,ω-bis*(*meta* dihydroxyphenyl) alkanes is now in progress.

EXPERIMENTAL

Preparation of 1,4-bis-(3,4-Methylene Dioxyphenyl)-butadiene-1,3.—Fifty grams of piperonal, 19.6 Gm. of succinic acid, and 74.3 Gm. of litharge were mixed with 93 cc. of acetic anhydride and boiled at 140° for five hours under a reflux condenser according to the technique of Kuhn (5). The mixture was cooled to 40° and pale yellow plate crystals were separated. These were collected together with the lead acetate which had also crystallized out on a Büchner filter. This crystalline mixture was washed with a small amount of acetic anhydride. The yellow crystals were washed free of lead acetate by means of water. The yellow crystals were then recrystallized several times from hot benzol, m. p. 221°. The yield was 12.1 Gm. or 24.7% of the theoretical.

Anal.—Calcd. for $C_{18}H_{14}O_4$: C, 73.47; H, 4.76. Found: C, 72.05; H, 4.82.

Preparation of 1,4-bis-(3,4-Methylene Dioxyphenyl)-butane.—The preparation of this compound by the catalytic reduction of the above compound offered considerable difficulties because of its extreme insolubility in all suitable solvents at 25°. No high pressure equipment was available which would permit using higher temperatures to dissolve the compound. No reduction was apparent when the compound was mixed with alcohol, platinum black, and hydrogen at 25° and 54 lb. pressure. Some reduction was obtained if the alcohol was warmed. However, the reduction apparatus was not constructed so as to keep the reaction warm, and, therefore, the amount reduced was quite limited. Some reduction was also obtained at about 50 lb. pressure by dissolving the unsaturated compound in a mixture of

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† Abstracted in part from a thesis submitted by Edward H. Carlson to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science.

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hot alcohol and acetone and using freshly prepared Raney nickel as a catalyst.

The catalyst was removed by filtration together with any undissolved material. The solution was then concentrated until upon cooling white needles were obtained. These upon recrystallization from alcohol melted at 79–80°.

Anal.—Calcd. for $C_{18}H_{18}O_4$: C, 72.48; H, 6.04. Found: C, 72.44; H, 6.72.

Preparation of 1,4-bis-(3,4-Dihydroxyphenyl)-butane.—Two grams of 1,4-*bis*-(3,4-methylenedioxyphenyl)-butane were mixed with 5.6 Gm. of phosphorous pentachloride and the mixture refluxed over a low flame for four and one-half hours. The volatile chlorides were removed by heating on the steam bath under vacuum and the residue hydrolyzed by boiling for an hour with 10% hydrochloric acid. The demethylenated product was dissolved in a hot mixture of methanol and water and decolorized with Norite. Upon cooling white crystals were obtained which melted at 140°. Recrystallization failed to raise the melting point. The demethylenated product was soluble in alkali, gave a negative test for methylene ethers and gave a green color with ferric chloride test solution.

Anal.—Calcd. for $C_{18}H_{18}O_4$: C, 70.07; H, 6.57. Found: C, 70.45; H, 6.96.

Preparation of 1,4-bis-(3,4-Dimethoxyphenyl)-butadiene-1,3.—This compound was prepared from veratric aldehyde and succinic acid by the procedure described for the preparation of 1,4-*bis*-(3,4-methylenedioxyphenyl)-butadiene-1,3. Poor yields of the *bis* compound were obtained from several attempts. It was obtained as pale yellow plates by crystallization from chloroform, m. p. 202–203°.

Anal.—Calcd. for $C_{22}H_{22}O_4$: C, 73.62; H, 6.75. Found: C, 73.37; H, 6.82.

Preparation of 1,4-bis-(3,4-Dimethoxyphenyl)-butane.—The above unsaturated compound 1,4-*bis*-(3,4-dimethoxyphenyl)-butadiene-1,3 was not very soluble even in hot ethanol and was reduced with Raney nickel at about 50 lb. pressure. The reduction product separated from hot ethanol upon cooling as white needles, m. p. 90°.

Anal.—Calcd. for $C_{20}H_{26}O_4$: C, 72.73; H, 7.88. Found: C, 72.40; H, 7.59.

Preparation of 1,4-bis-(3,4-Dihydroxyphenyl)-butane.—Six grams of 1,4-*bis*-(3,4-dimethoxyphenyl)-butane was dissolved in 20 cc. of glacial acetic acid and 4 cc. of acetic anhydride and 18 cc. of 48% hydrobromic acid were added. The yellow solution was refluxed for two hours. The solvents were removed under vacuum. The residue in the flask upon cooling became a crystalline solid mass. The demethylated product was crystallized twice from dilute methanol. White, flat, plate-like crystals that melted at 141–142° were obtained. A mixed melt with the demethylenated 1,4-*bis*-(3,4-methylene dioxyphenyl)-butane showed no depression. The demethylated product was soluble in alkali, gave a negative test for methoxyl groups, and gave a green color with ferric chloride test solution.

Preparation of 3-Methoxy-4-hydroxy Styryl Methyl Ketone.—To a cooled solution of 10 Gm. of sodium hydroxide in 100 cc. of water and 40 cc. of ethanol, 15.1 Gm. (0.10 mol.) of vanillin and 2.9 Gm. (0.05 mol.) of acetone were added in divided portions accompanied by vigorous stirring over a period of fifteen minutes (6). The mixture was stirred for an additional hour. The red-colored solution was then poured into a beaker containing 45 cc. of concentrated hydrochloric acid. Upon standing at 0° for a day, a yellowish orange crystalline material separated which was collected and crystallized from dilute ethanol to yield yellow crystals, m. p. 125–129°. Recrystallization from dilute ethanol yielded 2 Gm. of yellow crystals, m. p. 128–129°. The reported melting point of 3-methoxy-4-hydroxy styryl methyl ketone is 129°. No *bis* compound was obtained even though the limiting factors were changed. Therefore, the method of McGookin and Sinclair (7) was used to prepare working quantities of 3-methoxy-4-hydroxy styryl methyl ketone as an intermediate in the preparation of 3,3′-dimethoxy-4,4′-dihydroxy distyryl ketone: 43.79 Gm. (0.29 mol.) of vanillin, 81.2 cc. (1.4 mol.) of acetone, 34 cc. of ethanol, and 100 cc. of 20% sodium hydroxide solution were mixed in a 500-cc. flask. The mixture was kept at 0° for three days after which time the sodium salt of vanillin acetone separated. The sodium vanillin acetone was dissolved in a small amount of distilled water and carbon dioxide passed into it until precipitation was complete. The amorphous impure precipitate when dried weighed 45.0 Gm. or 80.5% of the theoretical. Recrystallization from 250 cc. of hot 50% ethanol yielded 36.5 Gm. (65.5% of theoretical) of yellow needles, m. p. 129°. The mother liquor upon concentration yielded 5 Gm. of impure material.

The benzyl ether was prepared according to Shriner and Fuson (8) and when crystallized first from acetone and then from ethanol melted at 91–92°. Reported melting point was 93°. The benzoate prepared by the Schotten–Baumann reaction gave colorless crystals from ethanol: m. p. 121–122°. Reported melting point, 121–122°.

Preparation of 3,3′-Dimethoxy-4,4′-dihydroxy Distyryl Ketone.—Attempts to prepare this compound according to the method of McGookin and Sinclair (7) using vanillin acetone and alkali yielded only small quantities of the *bis* compound. Therefore, McGookin and Sinclair's (7) acid catalyzed condensation of vanillin and acetone was used to give the desired results. One hundred grams of vanillin yielded 63.2 Gm. (55.49% of theoretical) of fine orange-red crystals. These crystals when recrystallized from 36% acetic acid yielded the monohydrate, m. p. 119–120° (reported 121°). Crystallization from a nonaqueous solvent such as chloroform or benzene yielded the anhydrous form, m. p. 141–142° (reported 142°).

Acetylation with acetic anhydride and sodium acetate yielded a canary-yellow diacetate when

crystallized from ethanol, m. p. 182–183° (reported 182°).

Benzoylation by means of the Schotten–Baumann reaction yielded yellow needles when crystallized from ethanol, m. p. 191–192° (reported 192°).

Preparation of 1,5-bis-(3-Methoxy-4-hydroxyphenyl) Pentan-3-one.—The distyryl ketone was readily reduced by Raney nickel in a Parr hydrogenation apparatus, using 3.26 Gm. (0.01 mol.) of the distyryl ketone, 0.6 Gm. Raney nickel, and 50 cc. of ethanol. The tetrahydro compound when recrystallized from 30% ethanol melted at 90–91° (reported 89.5–90.5°).

Anal.—Calcd. for $C_{19}H_{22}O_5$: C, 69.10; H, 6.66. Found: C, 68.92, 68.83; H, 6.53, 6.58.

A dibenzoate of the reduction product prepared by the Schotten–Baumann reaction was crystallized from ethanol, m. p. 131–132° (reported 129–130°).

A 2,4-dinitrophenylhydrazone was prepared as follows: 0.5 Gm. 2,4-dinitrophenylhydrazine, 0.5 Gm. of the saturated ketone, and 30 cc. of ethanol were diluted with water until the solution was almost turbid. One and two-tenths cubic centimeters of concentrated hydrochloric acid was added and the mixture refluxed for ten minutes. Upon cooling, an orange-red microcrystalline derivative was obtained, which upon recrystallization from hot ethanol melted at 122–125°.

Clemmensen Reduction of 1,5-bis-(3-Methoxy-4-hydroxyphenyl) Pentan-3-one.—This compound was reduced to 1,5-*bis*-(3-methoxy-4-hydroxyphenyl) pentane by a number of Clemmensen modifications. The best results were obtained when no solvent was used. Seventy-five per cent of theoretical were obtained of pure reduction products. Three and three-tenths grams (0.01 mol.) of 1,5-*bis*-(3-methoxy-4-hydroxyphenyl) pentan-3-one, 9.0 Gm. of amalgamated zinc, 20 cc. of concentrated hydrochloric acid, and 8.0 cc. of water were refluxed for fifteen hours. A greenish oil solidified on the surface of the reaction mixture after cooling. Recrystallization from 20% ethanol yielded white plates, m. p. 70–71°. When crystallized from 10% acetic acid, small white needles were obtained.

Anal.—Calcd. for methoxyl, $C_{17}H_{18}O_2(OCH_3)_2$: 19.6. Found: 19.72, 19.81.

A dibenzoate was prepared by the Schotten–Baumnn reaction which when crystallized from ethanol melted at 114–115°.

Preparation of 1,5-bis-(3,4-Dihydroxyphenyl) Pentane.—Demethylation of 1,5-*bis*-(3-methoxy 4-hydroxyphenyl) pentanewas accomplished as follows: 16.4 Gm. of 1,5-*bis*-(3-methoxy 4-hydroxyphenyl) pentane, 67.7 Gm. of glacial acetic acid, and 26 Gm. of constant boiling hydrobromic acid were refluxed for six hours under a constant stream of carbon dioxide gas. After twenty minutes, the reaction mixture became purplish blue in color. After six hours the acid solution was neutralized with a saturated sodium bicarbonate solution. An oil that separated was placed in the refrigerator for two days after which time white crystalline platelets were obtained. The crystals were removed from the residual oil by suction in a Büchner funnel. They were crystallized from 400 cc. of 10% acetic acid to yield 11 Gm. of white platelets, m. p. 126–129°. Recrystallization from 10% acetic acid raised the m. p. to 131–132°.

A dilute hydroalcoholic solution of these platelets gave a green color with ferric chloride test solution. Dropwise addition of a dilute solution of sodium carbonate caused the following color changes, dark green, blue, purple, and finally a purplish red. These color changes are characteristic of catechol groups.

A quantitative determination for methoxyl groups by the Zeissel method indicated the absence of methoxyl groups.

Benzoylation by the Schotten–Baumann process yielded a derivative that was recrystallized from methanol, m. p. 89–91°.

Preparation of 1,4-Diveratroyl Butane.—Eighty-five grams of veratrole and 400 cc. of dry carbon disulfide were placed in a one-liter, three-necked flask equipped with a mercury-sealed stirrer, reflux condenser, and a dropping funnel. This mixture was well cooled and, while being stirred, 96.7 Gm. of anhydrous powdered aluminum chloride was added in small portions. Some of the aluminum chloride separated as a gummy mass. Adipyl chloride was added *via* the dropping funnel at an even rate during ninety minutes. The mixture was stirred rapidly during the above addition and the reaction flask was immersed in an ice bath. The reaction mixture became tarry and black. The ice bath was removed and the mixture stirred for three hours. As the reaction mixture warmed to room temperature, more tar was formed and stirring became increasingly difficult. The tarry reaction mixture was decomposed with 500 Gm. of cracked ice and 100 cc. of concentrated hydrochloric acid. After standing overnight the mixture was poured into a separatory funnel and the carbon disulfide layer separated from the acid-aqueous layer. The acid-aqueous layer, which contained some insoluble matter, was extracted with hot benzene. The hot benzene solution was treated with several portions of 5% aqueous sodium carbonate to remove any acidic constituents. One of these acidic constituents was later shown to be δ-veratroyl valeric acid. The benzene solution was concentrated and upon cooling deposited crystalline material. Forty-six grams of fine needle-like crystals were obtained from the benzene solution. Several recrystallizations from ethanol gave five white needles, m. p. 149–150°.

Anal.—Calcd. for $C_{22}H_{26}O_6$: C, 68.40; H, 6.71. Found: C, 68.26; H, 6.79.

The adipyl chloride used in the above synthesis was prepared according to "Organic Synthesis" (9). The veratrole was prepared by methylating guaiacol with dimethyl sulfate and alkali. Ninety-two per cent to 95% yields of veratrole were obtained that boiled at 204°.

The δ-veratroyl valeric acid obtained by the acidification of the sodium carbonate washings was dis-

solved in ethanol and decolorized with charcoal. It was best crystallized from toluene to obtain crystals that were finally recrystallized from benzene, m. p. 122–123°.

Anal.—Calcd. for $C_{14}H_{18}O_5$: C, 63.91; H, 6.77. Found: C, 63.26; H, 7.21.

Preparation of 1,6-Diveratryl Hexane.—Considerable difficulty was encountered in determining the right conditions for a Clemmensen reduction of the 1,4-diveratroyl butane. The following procedure gave the best results. Ten grams of 1,4-diveratroyl butane was placed in a 250-cc. round-bottomed flask equipped with a reflux condenser. Fifty grams of amalgamated zinc, 54 cc. of 20% hydrochloric acid, 17 cc. of toluene, and 10 cc. of glacial acetic acid were added. This mixture was refluxed for twenty hours over a low flame. The toluene was separated from the aqueous acid layer. The acid aqueous layer was extracted several times with ether and combined with the toluene layer. This solution was washed free of acid with water, and dried over anhydrous sodium sulfate. The solvents were removed by distillation. A yellow oil was obtained that was warmed with an equal volume of methanol and then placed in the refrigerator. Six and seven-tenths grams of crystalline material was obtained. Several recrystallizations from ethanol gave a crystalline product that melted at 76.5–77.5°.

Anal.—Calcd. for $C_{22}H_{30}O_4$: C, 73.74; H, 8.38. Found: C, 73.92; H, 8.61.

Preparation of 1,6-bis-(3,4-Dihydroxyphenyl) Hexane.—Four and one-half grams of 1,6-diveratryl hexane, 15 cc. of glacial acetic acid, 3 cc. of acetic anhydride, and 15 cc. of 48% hydrobromic acid were refluxed for two hours in a 100-cc. round bottomed flask. The solvents were removed by distillation under a vacuum. The crude material was dissolved in hot toluene, filtered, and placed in the refrigerator overnight. Rosette-like crystals slowly formed and were recrystallized from 12% acetic acid. Yield 1.5 Gm., m. p. 128–129°.

Anal.—Calcd. for $C_{18}H_{22}O_4$: C, 71.52; H, 7.28. Found: C, 71.26; H, 7.74.

This compound gave a green color with ferric chloride that changed to blue to violet and finally wine-red upon the dropwise addition of a very dilute solution of sodium carbonate. This color reaction is characteristic of catechol groups.

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Book Reviews

New Drugs, by ARTHUR D. HERRICK, with brief foreword by AUSTIN E. SMITH, M.D. Revere Publishing Co., New York. 1946. xiv + 303 pp., 6 x 9, buckram. Price $4.00.

As set forth in the preface the author has "endeavored to present . . . the important phases of new drug practice and procedure, and to explain the requirements of the law and regulations so that these may be applied to a particular product."

In the foreword Dr. Austin E. Smith, Secretary of the Council on Pharmacy and Chemistry of the American Medical Association, comments: "The purpose of this book is to provide the information which will enable those concerned in any way with drug therapy to become acquainted with the responsibilities involved in the release of new drugs to the public."

It is pointed out in the preface that the administrative interpretations of Section 505 of the Food Drug and Cosmetic Act by the Food and Drug Administration have changed somewhat from time to time as experience with the Act has accumulated and the book appears to present an up to date interpretation by reason of the fact that it is stated to have been read in manuscript by several members of the Food and Drug Administration, particularly Dr. Walton Van Winkle, who until recently was directly in charge of all new drug applications.

To those who have had little or no experience with the preparation and presentation of new drug applications to the Food and

Drug Administration this book should prove very helpful, but for those with considerable such experience it presents nothing new, as may well be expected. The book would be excellent as required reading in a college course on the legal aspects of Pharmacy, especially the first 98 pages.

It will not answer all the questions which may arise in the course of preparing any particular new drug application nor should this be expected, but it does give a substantial background of information. Since the author is a lawyer it is natural that the legal viewpoint predominates but it seems unnecessary that so much space is given to legal discussion of certain phases of the law. For example 55 pages are used to discuss "Refusal to Permit Effectiveness," "Refusal Proceedings," "Revocation of Refusal," "Suspension of Effective Application," and "Appeals from Orders." About 90 pages actually deal with the essential requirements of a new drug application. The first chapter (7 pages) deals with the origin of Section 505 of the Act.

The four appendices cover 136 pages and it is of questionable value to use 44 pages in Appendix A to reprint Senate Document No. 127, 75th Congress, 2nd Session, which is the report on the Sulfanilamide Elixir investigation, even though of some historical interest in the development of the new drug section of the law.

Appendix B gives the full text of the Food Drug and Cosmetic Act including the recent sections on Penicillin and Insulin, also the general regulations. This is desirable for ready reference. It is unfortunate however that a proper footnote does not indicate that the very extensive regulations concerning 502(d), Derivatives of Habit-forming Drugs; 406(b) 504, 604, and 706, all pertaining to Coal Tar Colors; 506, Insulin; and 507, Penicillin, are not included when such indication is given in various other less important places.

Appendix C (14 pages) is useful in its brief record of certain city and state laws concerning new drugs.

Appendix D reprints in 26 pages the rules of the Council on Pharmacy and Chemistry of the American Medical Association. This is interesting and useful but of course has no direct bearing on the Food, Drug, and Cosmetic Act.

The index is good and will facilitate materially the proper use of the book.—FRANK O. TAYLOR

Soil and Plant Analysis, by C. S. PIPER. Interscience Publishers, Inc., New York.

This monograph by Dr. C. S. Piper, Chemist at the Waite Agricultural Research Institute, of the University of Adelaide, South Australia, has as its secondary title, "A Laboratory Manual of Methods for the Examination of Soils and the Determination of the Inorganic Constituents of Plants." Many chemists when called upon to undertake the examination of soils or plant materials are confronted with a vast array of methods in the literature. Frequently these methods are of an empirical nature, adaptable only to certain problems, and when adopted for entirely different soil types yield only erroneous and conflicting results. The author describes several methods in use at his own and other agricultural experiment stations.

Part I which deals with the examination of soils, describes methods for the collection of samples, the determination of *p*H, conductivity and water-soluble salts, mechanical analysis, single value soil constants, color calcium carbonate, exchangeable ions and exchange capacity, nitrogen, nitrates, nitrites and ammonia, organic matter, etc. Part II discusses methods for the determination of the inorganic constituents of plants; methods for the collection of samples, for ashing, for the determination of the more common inorganic constituents as silica, calcium and magnesium, potassium, sodium, phosphorous, chlorine and sulfur and for the determination of the trace elements, boron, cobalt, copper, iron, manganese, molybdenum and zinc. Many references to the literature are also given.

The methods are excellently presented. The book, while of great value to experimental horticulturists, fills a long-felt want among pharmacognosists working in the field of drug plant cultivation.—ELMER H. WIRTH.